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SIMON FLEXNER, M.D. PEYTON ROUS, M.D.

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BLOOD DESTRUCTION DURING EXERCISE.

I. Blood Changes Occurring in the Course of a Single Day of Exercise.

By G. O. BROUN, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

(Received for publication, June 13, 1922.)

The studies of Rous and Robertson[1] have shown that normally the destruction of erythrocytes is accomplished, in part at least, by a process of fragmentation in the blood stream. There is evidence that the breaking up is the result of mechanical injury. Among physiologic conditions which would favor such disintegration, vigorous exercise at once suggests itself. Both the increased rapidity of blood flow and the violent muscular contractions would tend to increase the wear and tear upon the corpuscles. The present investigations have been undertaken with the object of determining whether an increased rate of blood destruction during exercise could be demonstrated.

Since the subject is closely linked with the general question of fluid and cell changes during exercise, a number of observations have been made in this connection, and it is with these that the present paper will be largely concerned. The data given serve as controls to the method employed in experiments subsequently to be reported.

The changes in the blood during exercise are not yet completely understood. The early literature upon the subject has been well summarized by Hawk.[2] Practically without exception, investigators have reported an increase in hemoglobin and corpuscles immediately after short periods of exercise. Schneider and Havens[3] confirmed these findings in observations made at low altitudes, but following exercise at high altitudes found no increase in either cells or pigment. On the

[1] Rous, P., and Robertson, O. H., *J. Exp. Med.*, 1917, xxv, 651.

[2] Hawk, P. B., *Am. J. Physiol.*, 1903–04, x, 384.

[3] Schneider, E. C., and Havens, L. C., *Am. J. Physiol.*, 1914–15, xxxvi, 380.

other hand, Cohnheim and his coworkers[4] as well as Gross and Kestner[5] have shown an actual decrease in red count and per cent of hemoglobin after long and strenuous mountain climbing. This they attribute to dilution of the blood with fluid derived from the muscles. The workers who have found a concentration of the blood during exercise explain it either by a changed distribution of the corpuscles in the vessels[2] or by loss of fluid into the muscles[6] or liver lymphatics.[7]

Obviously the points at issue can only be cleared up by accurate determinations of cell and plasma volume during exercise. So far as we are aware, no such observations have been reported. The lack of a method of estimating blood volume which is at once accurate and innocuous has no doubt hitherto prevented advancement along this line.

Method.

Two types of blood volume determination have shown themselves to be of practical value for use on the living animal. The first is the well known carbon monoxide inhalation method, originally devised by Gréhant and Quinquaud.[8] The second entails the addition of some substance to the blood plasma and the subsequent determination of its degree of dilution. Perhaps the most satisfactory of the numerous substances employed in this way is the dye, vital red, first used by Keith, Rowntree, and Geraghty.[9] The non-toxic nature of the dye allows repetition of the volume determinations at short intervals. Since the carbon monoxide method cannot readily be repeated within a single day, the dye method was adopted for use in the present series of experiments.

The technique followed closely that described in detail by Hooper, Smith, Belt, and Whipple[10] and Smith.[11] Mention need be made only of slight modifications.

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[4] Cohnheim, O., Kreglinger, and Kreglinger, *Z. physiol. Chem.*, 1909, lxiii, 413.

[5] Gross, W., and Kestner, O., *Z. Biol.*, 1920, lxx, 187.

[6] von Willebrand, E. A., *Skandin. Arch. Physiol.*, 1903, xiv, 176.

[7] Lamson, P. D., *J. Pharmacol. and Exp. Therap.*, 1920–21, xvi, 125.

[8] Gréhant and Quinquaud, E., *Compt. rend. Acad.*, 1882, xciv, 1450.

[9] Keith, N. M., Rowntree, L. G., and Geraghty, J. T., *Arch. Int. Med.*, 1915, xvi, 547.

[10] Hooper, C. W., Smith, H. P., Belt, A. E., and Whipple, G. H., *Am. J. Physiol.*, 1920, li, 205.

[11] Smith, H. P., *Am. J. Physiol.*, 1920, li, 221.

Vital red was used in 1 per cent strength for injection, and dogs of 10 to 15 kilo body weight received 4 cc. of it, while those above 15 kilos received 5 cc. In making the dilute dye solution for the preparation of a standard, 1 cc. of the 1 per cent dye solution was made up with distilled water to 250 cc. in a volumetric flask. This gave a solution of the strength of 1/25,000, a figure easier to deal with than the fraction 1/26,666.67 employed in the articles referred to above. It was found to be quite important to allow the blood to resume its normal flow for several minutes after the manipulations necessary to the introduction into the vein of the aspirating needle, since stasis caused by pressure on the vessel changes the hematocrit value. The procedure employed was as follows:

The needle, firmly grasped at the base by a long jawed hemostat, is connected to a syringe containing a little saline solution. While an assistant makes pressure upon the external jugular vein just above the clavicle, the needle is thrust into its lumen. The aspiration of blood into the saline solution shows clearly when the needle is in place. Pressure on the vein is then discontinued, the syringe is detached, leaving the needle in place, and a close fitting stylet is introduced into the lumen of the latter, completely closing it. After 2 or 3 minutes have been permitted to elapse, during which presumably the normal flow in the vein is completely reestablished, the stylet is withdrawn and a sample of blood taken into a syringe. The same method is used in obtaining both specimens required for each volume determination. In the case of the second specimen it has the special advantage of insuring the accuracy of the 4 minute period between the dye injection and the withdrawal of the blood.

The hematocrit determinations were made by filling three tubes of the type described by Epstein[12] from each specimen of blood and centrifuging these at 3,500 revolutions per minute for 30 minutes. The average plasma per cent of the three tubes, after correction for oxalate dilution, was taken as the true plasma per cent of the blood. To obtain the plasma for colorimetric readings, the centrifuge tubes were centrifuged at 2,000 revolutions per minute for 10 to 15 minutes. This modification, while adding somewhat to the complexity of the method, has given more uniform results than the use of graduated centrifuge tubes.

Hemoglobin determinations were made by the method of Newcomer.[13] The colored glass standard had been checked against gasometrically standardized Palmer[14] and Robscheit[15] solutions and found to correspond closely to these throughout a wide range. The specimen for hemoglobin was withdrawn from the oxalated blood of the jugular vein as obtained for the volume determinations. The reading was subsequently corrected for dilution of blood with oxalate. Per cent of hemoglobin is given in terms of the Haldane scale. 100 per cent hemoglobin on this

[12] Epstein, A. A., *J. Lab. and Clin. Med.*, 1915–16, i, 610.
[13] Newcomer, H. S., *J. Biol. Chem.*, 1919, xxxvii, 465.
[14] Palmer, W. W., *J. Biol. Chem.*, 1918, xxxiii, 119.
[15] Robscheit, F. S., *J. Biol. Chem.*, 1920, xli, 209.

scale is equivalent to an oxygen-carrying capacity of 18.5 cc. per 100 cc. of whole blood. This corresponds to 13.8 gm. of hemoglobin per 100 cc. of whole blood.

The few red counts recorded were made in duplicate from the oxalated blood of the jugular vein, subsequently corrected for oxalate dilution. Hayem's fluid was used and the Bürker-Zeiss counting chamber.

In reporting the results the term "pigment volume" has been used in the sense in which Whipple and his coworkers[16] employed it; namely, as the product of the blood volume by the per cent of hemoglobin. Subject to the obvious limitations of the vital red method, it will serve as an index of the total circulating blood pigment. But since the hematocrit and hemoglobin values of blood specimens withdrawn from the jugular vein are influenced by changes in cell distribution, this factor must be borne in mind in interpreting the results.

Experimental Procedure.

The chief reason for choosing dogs as the experimental animals, apart from their general utility, was the special susceptibility of their red corpuscles to mechanical injury.[17] It seemed probable that because of this characteristic the blood destruction during exercise would be greater than in the other available species.

The animals were kept in individual cages and fed upon a mixed diet containing considerable meat. To avoid possible changes in blood volume resulting from the ingestion of food, food was not given upon the day of the experiment until after the last blood volume had been completed. But it was found necessary to allow water freely.

For purposes of exercise, treadmills were used, so constructed that the tread was at an angle of 20° to the horizontal. A bicycle cyclometer attached to the machine recorded the number of miles traveled. The standard day's exercise consisted of two periods, each of 2 hours, separated by a rest of 1 hour. The distances covered during these periods varied considerably, being chiefly limited by the ability of the animals to withstand the exertion. All of the animals worked actively, yet the average per day for the series was only 3.8 miles. This may

[16] Whipple, G. H., Hooper, C. W., and Robscheit, F. S., *Am. J. Physiol.*, 1920, liii, 151.

[17] Rous, P., and Turner, J. R., *J. Exp. Med.*, 1916, xxiii. 219.

seem surprisingly low, but the fact should be recognized that the 20° inclination of the tread necessitated a vertical ascent of 6,870 feet in this distance. A careful oversight was maintained to prevent exhaustion. Except in the initial period of 10 to 15 minutes exercise, no attempt was made to insure a continuous run, brief rest being allowed as needed. However, immediately before the last of the three blood examinations, the animal was run continuously for about 10 minutes.

It should be noted that the twelve complete experiments here reported were all done during warm weather and panting was regularly induced.

Two series of experiments were performed which will be designated respectively the "exercise series" and the "control series."

Three animals, Nos. 3, 4, and 7, appear in both series of experiments, and in the exercise series Nos. 3 and 5 appear twice. The repetition of experiments on the same animal was in most cases carried out only after the lapse of a considerable time, and during the interval some change in body weight occasionally occurred. The experiments on No. 8 and the first of the two on No. 5 were made when the animals had not completely recovered from an anemia induced by an hemolytic serum. The conditions in these cases were not entirely normal, yet the blood changes noted are of the same general character as those of the other animals of the series. We have, however, deemed it advisable to list the results with them separately in Table I, which shows the "normal" blood findings of the animals. All of the other animals were in good health at the time of observation.

The exercise experiments were conducted in the following manner. The dog was removed directly from cage to table and the first blood volume specimens were taken. From 15 to 20 minutes later the animal was placed on the treadmill and exercised vigorously for 10 to 15 minutes, then quickly removed, and a second set of determinations on the blood made at once. Following this the animal was exercised for 2 hours, allowed to rest for 1 hour, and again exercised for another 2 hour period. Finally, at the end of this time, the third blood volume determination was made. This procedure was slightly altered in the case of Dogs 1 and 7 which were exercised for 3 consecutive hours between the second and third blood volume determinations, without rest or access to water. In the first experiment on Animals 3 and 5, and in the single experiment on Animals 6 and 8, the blood was examined before exercise and after 10 minutes exercise. A third examination was not made.

In the control series the three blood examinations were made at approximately the same intervals as in the exercise series. In the case of Dogs 10 and 3, only two determinations were carried out, an interval of 30 minutes separating those of Dog 10 and 6 hours those of Dog 3.

TABLE I.

Blood Findings at Initial Examination.

Series.	Animal No.	Body weight.	Plasma volume.	Plasma per 100 gm. of body weight.	Cell volume.	Cells per 100 gm. of body weight.	Entire blood volume.	Blood volume per 100 gm. of body weight.	Hemoglobin per 100 gm. of body weight.	Remarks.
		kg.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*gm.*	
Exercise series.	1	20½	1,078	5.25	782	3.82	1,860	9.08	1.39	
	2	14½	923	6.36	717	4.95	1,640	11.30	1.85	
	3	20¼	1,244	6.14	936	4.62	2,180	10.75		6 days after control observation noted below.
	3	18¼	1,190	6.53	968	5.30	2,158	10.62	1.93	3 mos. later still.
	4	18¾	1,226	6.54	818	4.36	2,044	10.90	1.70	3 " before control observations noted below.
	5	14	940	6.71	408	2.91	1,348	9.64	1.18	2 mos. after observations noted below.
	6	20¾	983	4.74	967	4.66	1,950	9.40		
	7	18½	1,030	5.58	812	4.39	1,842	9.96	1.54	3 mos. before control observations noted below.
Control series.	9	11¾	703	5.98	507	4.31	1,210	10.30	1.68	
	10	14¼	719	5.04	723	5.07	1,442	10.11	1.77	
	11	14¾	736	4.99	563	3.82	1,299	8.80	1.35	
	12	11¾	692	5.89	610	5.19	1,302	11.10	2.08	
	3	20¼	1,174	5.80	894	4.41	2,068	10.20		6 days before first exercise observation.
	4	20¾	1,402	6.77	683	3.29	2,058	9.91	1.20	3 mos. after exercise observation.
	7	16¼	998	6.13	723	4.45	1,721	10.60	1.67	3 " " " "
Average.....				5.89		4.37		10.18	1.61	

We have purposely omitted the following blood volume determinations of Animals 5 and 8 from the normal series as the animals had not entirely recovered from anemia due to the injection of an hemolytic serum. Their reactions to exercise did not differ from those of normal animals.

	5	14	941	6.72	328	2.34	1,269	9.06		2 mos. previous to observation noted above.
	8	$10\frac{1}{4}$	849	8.29	405	3.95	1,254	12.24		

The Blood in the Resting Animal.

The findings in the initial blood specimen in both series of animals are given in Table I. This specimen is assumed to yield the normal relation of the blood components during rest. The figures obtained compare closely with those reported for normal dogs by Smith, Arnold, and Whipple.[18] The average cell volume is somewhat smaller and the plasma volume slightly larger than they found by the use of the dye method. The cell volume figures, however, correspond almost exactly to their determinations made by the carbon monoxide method, which they regard as yielding figures that closely approximate the true cell volume. The accompanying high hemoglobin content of the blood shows that the differences noted are not due to anemia.

The Effect of Brief Exercise.

The changes which occurred during exercise are recorded in Table II and are graphically shown in Text-fig. 1. Since the first examination of the blood is taken to indicate the normal condition in each animal while resting, there is shown in the table to the right of the actual figure for each determination its percentage in terms of the findings at the first determination. In Text-fig. 1 the percentage changes in each of the blood components are shown and compared with the initial finding.

It will be noted that 10 to 15 minutes of active exercise caused usually a slight increase in plasma volume and quite regularly a marked increase in cell volume, hemoglobin, pigment volume, and number of corpuscles per cubic millimeter. For the series there is an average increase in plasma of 3.6 per cent. In two individuals it showed a slight decrease. The cell volume increased in every case, averaging 12.3 per cent, with a variation from 1.5 per cent to 24.0 per cent. Pigment volume follows closely the changes in cell volume, the increase ranging from 5 to 25 per cent with an average of 16.1 per cent. The per cent of hemoglobin shows a maximal rise of 13 and a minimal of 2, the average being 8.5. The increase in number of red corpuscles per cubic millimeter averages 11 per cent for the two instances followed.

[18] Smith, H. P., Arnold, H. R., and Whipple, G. H., *Am. J. Physiol.*, 1921, lvi, 336.

The Changes Consequent on More Prolonged Exercise.

After several hours of exercise, the plasma volume shows consistently an increase. The average rise above the resting normal is 11.7 per cent, with minimal and maximal variations of 6.8 and 18 per cent. The average increase over the plasma volume after 10 minutes of exercise is 8.1 per cent. In one instance there is a decrease in plasma volume, but of less than 1 per cent, between the second and third determinations. The greatest increase observed is 13 per cent. On the other hand, cell volume, while remaining at an average of 9.4 above the resting cell volume, shows an average decrease of 2.9 per cent below the average found at the end of 10 minutes exercise. In two instances there are increases of 1 and 2.5 per cent. The tendency of the hemoglobin percentage to decrease is more marked but this is, in part at least, due to dilution of the blood by increase in plasma volume. However, the calculated pigment volume, in which this error of dilution is corrected, shows after the period of prolonged exercise a 7.7 per cent fall below the average after 10 minutes of exercise. In no instance is an increase to be found. The final average pigment volume is, however, 8.4 per cent above the average of the first determination. The red count in the two instances followed shows a marked decrease after the prolonged exercise, part of which may be ascribed to the dilution of the blood referred to above.

The Control Series.

Although the animals used as controls were kept in small cages throughout the periods of observation, it was not possible to prevent a certain amount of activity on their part. The dogs varied much in this respect. It is not surprising, then, that the results obtained vary somewhat too (see Table III and Text-fig. 2). Nevertheless they are in striking contrast with those of the exercise series (compare Tables II and III). There is an average increase of 4.6 per cent in the second plasma volume over the first, an increase which closely corresponds to that observed in animals exercised for 10 minutes. It is quite possible that in both series a portion of this increase may be due to the disturbing influence of the first blood volume determination, in which approximately 20 cc. of blood is withdrawn and 4 to 5 cc.

TABLE II.

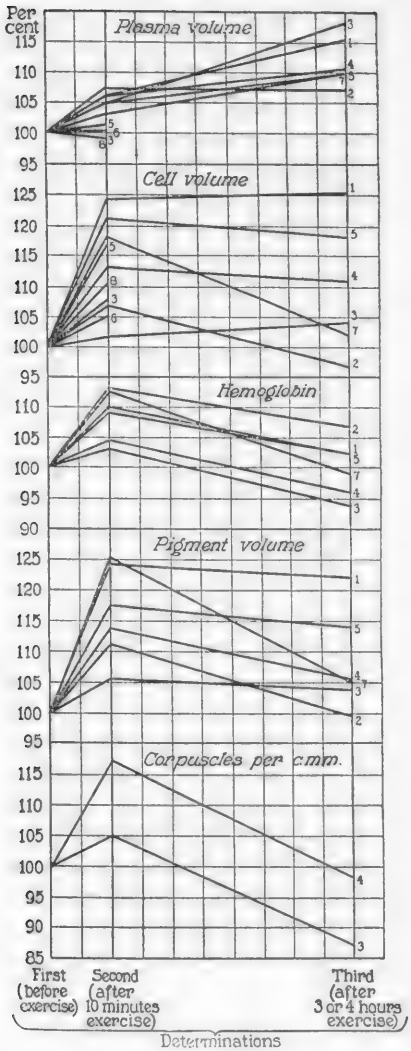
The Changes during Exercise.

Animal.	Elapsed time.	Circumstances.	Plasma volume.		Cell volume.		Hemoglobin.		Pigment volume.		Red corpuscles.	
			Cc.	In percentage of the first plasma volume here given.	Cc.	In percentage of the first cell volume here given.	Per cent.	In percentage of the first hemoglobin determination here given.	Units.	In percentage of the first pigment volume here given.	Per c. mm.	In percentage of the first red count here given.
1. Female collie; weight 20½ kilos.	0	Before exercise.	1,078	100.0	782	100.0	111.0	100.0	2,062	100.0		
	30 min.	After 10 min. exercise.	1,140	106.0	970	124.0	122.0	110.0	2,570	124.0		
	3 hrs., 30 min.	After 3 hrs. exercise. Had run 3 miles. No rest period.	1,242	115.0	979	125.0	113.0	102.0	2,512	122.0		
2. Male mongrel; weight 14½ kilos.	0	Before exercise.	923	100.0	717	100.0	119.0	100.0	1,950	100.0		
	30 min.	After 10 min. exercise.	992	107.5	764	106.5	123.0	113.0	2,160	111.0		
	3 hrs., 30 min.	After 4 hrs. exercise. Had run 4 miles in all with an interpolated hr. of rest.	985	106.8	691	96.5	116.0	106.5	1,944	99.8		

3. Male foxhound; weight 20¼ kilos.	0	Before exercise.	1,244	100.0	936	100.0						
	30 min.	After 10 min. exercise.	1,234	99.5	1,006	107.5						
	0	Before exercise.	1,190	100.0	968	100.0	118.0	100.0	2,548	100.0	8,600,000	100.0
	30 min.	After 10 min. exercise.	1,249	105.0	981	101.5	120.5	103.2	2,690	105.5	9,000,000	105.0
3 mos. later; weight 18¼ kilos.	5 hrs., 30 min.	After 4 hrs. exercise. Had run 5 miles in all with an interpolated hr. of rest.	1,405	118.0	1,005	104.0	110.0	93.5	2,650	104.0	7,500,000	87.3
4. Male pointer; weight 18¾ kilos.	0	Before exercise.	1,226	100.0	818	100.0	113.0	100.0	2,310	100.0	6,100,000	100.0
	30 min.	After 10 min. exercise.	1,298	106.0	922	113.0	118.0	104.5	2,620	113.5	7,130,000	117.0
	5 hrs., 30 min.	After 4 hrs. exercise. Had run 5 miles in all with an interpolated hr. of rest.	1,352	110.5	909	111.0	108.0	95.7	2,440	105.5	6,000,000	98.5
5. Male collie; weight 14 kilos.	0	Before exercise.	941	100.0	328	100.0						
	5 hrs.	After 10 min. exercise.	955	101.5	382	116.5						
	0	Before exercise.	940	100.0	408	100.0	89.0	100.0	1,200	100.0		
	30 min.	After 10 min. exercise. Had a 1 hr. rest period at noon.	965	103.0	493	121.0	96.8	109.0	1,410	117.5		
3 mos. later; weight unchanged.	5 hrs., 30 min.	After 4 hrs. exercise. Had run 3 miles.	1,033	110.0	483	118.0	90.5	102.0	1,372	114.0		

TABLE II—*Concluded.*

Animal.	Elapsed time.	Circumstances.	Plasma volume.		Cell volume.		Hemoglobin.		Pigment volume.		Red corpuscles.	
			Cc.	In percentage of the first plasma volume here given.	Cc.	In percentage of the first cell volume here given.	Per cent.	In percentage of the first hemoglobin determination here given.	Units.	In percentage of the first pigment volume here given.	Per c. mm.	In percentage of the first red count here given.
6. Male setter; weight 20½ kilos.	0	Before exercise.	983	100.0	967	100.0						
	30 min.	After 10 min. exercise.	990	100.5	1,025	105.0						
7. Male mongrel; weight 18½ kilos.	0	Before exercise.	1,030	100.0	812	100.0	112.0	100.0	2,062	100.0		
	30 min.	After 10 min. exercise. No rest period.	1,080	105.0	960	118.0	126.0	112.5	2,570	125.0		
	3 hrs., 30 min.	After 3 hrs. exercise. Had run 3 miles.	1,132	110.0	830	102.0	110.0	98.5	2,160	105.0		
8. Male bull mongrel; weight 10⅕ kilos.	0	Before exercise.	849	100.0	405	100.0						
	4 hrs.	After 10 min. exercise.	845	99.5	447	110.5						



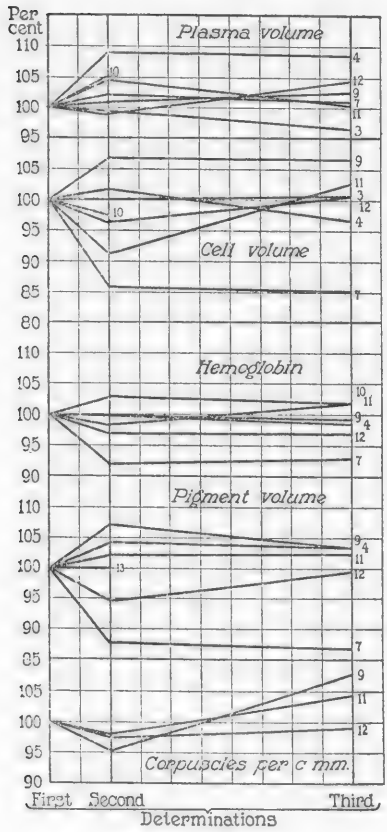
TEXT-FIG. 1. Percentage changes in the blood components during exercise.

TABLE III.

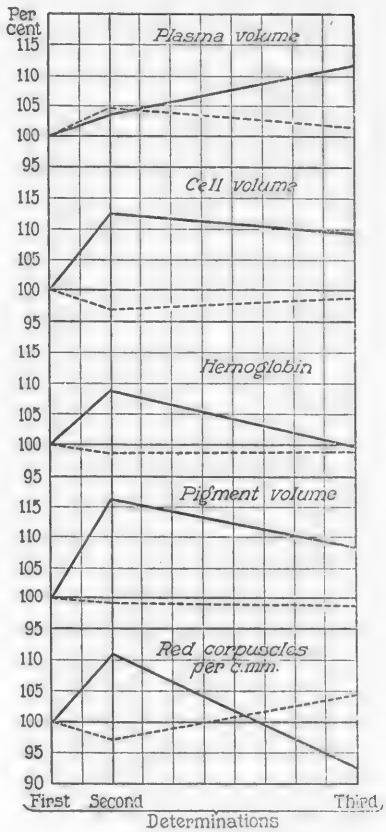
Control Findings.

Animal.	Elapsed time.	Circumstances.	Plasma volume.		Cell volume.		Hemoglobin.		Pigment volume.		Red corpuscles.	
			Cc.	In percentage of the first plasma volume here given.	Cc.	In percentage of the first cell volume here given.	Per cent.	In percentage of the first hemoglobin determination here given.	Units.	In percentage of the first pigment volume here given.	Per c. mm.	In percentage of the first red count here given.
9. Male mongrel; weight 11¾ kilos.	0	No exercise on this day.	703	100.0	507	100.0	118.0	100.0	1,437	100.0	8,000,000	100.0
	30 min.		708	100.8	544	107.0	119.0	100.9	1,490	104.0	7,600,000	95.0
	5 hrs., 30 min.		717	102.0	543	107.0	117.5	99.6	1,480	103.0	8,600,000	107.2
10. Male mongrel; weight 14¼ kilos.	0	No exercise on this day.	719	100.0	723	100.0	127.0	100.0	1,832	100.0		
	30 min.	Only two blood volume determinations made.	752	105.0	716	97.7	125.0	98.5	1,832	100.0		
	5 hrs., 30 min.						130.0	102.0				
11. Male mongrel; weight 14¾ kilos.	0	No exercise on this day.	736	100.0	563	100.0	111.0	100.0	1,440	100.0	7,800,000	100.0
	30 min.		770	104.5	514	91.5	114.0	103.0	1,465	102.0	7,600,000	97.5
	5 hrs., 30 min.		730	99.8	581	103.0	112.0	102.0	1,490	102.0	8,100,000	103.8

12. Male mongrel; weight 11¾ kilos.	0	No exercise on this day.	692	100.0	610	100.0	134.0	100.0	1,748	100.0	8,200,000	100.0
	30 min.		682	99.0	588	96.5	130.0	97.1	1,652	94.5	8,000,000	97.6
	5 hrs., 30 min.		724	104.0	614	101.0	130.0	97.1	1,740	99.5	8,100,000	98.8
3. Male foxhound; weight 20¼ kilos.	0	No exercise on this day.	1,174	100.0	894	100.0						
	6 hrs.	Only two determinations made.	1,127	96.0	911	101.0						
4. Male pointer; weight 20¾ kilos.	0	No exercise on this day.	1,402	100.0	683	100.0	88.0	100.0	1,835	100.0		
	1 hr.		1,536	109.0	696	102.0	88.0	100.0	1,960	107.0		
	5 hrs.		1,510	108.0	665	97.0	87.0	99.0	1,890	103.0		
7. Male mongrel; weight 16¼ kilos.	0	No exercise on this day.	998	100.0	723	100.0	114.0	100.0	1,965	100.0		
	1 hr.		1,020	102.0	620	86.0	105.0	92.2	1,722	88.0		
	5 hrs.		985	100.0	613	85.0	106.0	93.0	1,705	87.0		



TEXT-FIG. 2.



TEXT-FIG. 3.

TEXT-FIG. 2. Control findings. The animals were confined, throughout, to small cages.

TEXT-FIG. 3. Average changes in the blood components in the two series.

——— Exercise series.
- - - - Control series.

of dye solution injected. Cell volume shows a marked decrease in four animals, a slight increase in one, and a considerable increase in another. The average for the series is a decrease of 3.2 per cent. Hemoglobin and pigment volume show irregular changes mostly within rather narrow limits. The red count also decreases slightly. The third set of determinations shows irregular variations in all the blood components but within narrow limits, and the average is very close to the normal, taking the results of the first determination as such.

In Table IV, the changes in both series of animals have been compared. Only the average variations in per cent of the initial or "normal" average are there given. These percentage changes are also made the basis of the graphic representation in Text-fig. 3 where

TABLE IV.

Summary of the Average Changes in the Two Series.

Determination.	Plasma volume.		Cell volume.		Hemoglobin.		Pigment volume.		Red corpuscles per c. mm.	
	Exercise series.	Control series.	Exercise series.	Control series.	Exercise series.	Control series.	Exercise series.	Control series.	Exercise series.	Control series.
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
First.	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Second.	103.6	104.6	112.3	96.8	108.5	98.6	116.1	99.2	111.0	97.1
Third.	111.7	101.6	109.4	99.0	99.6	98.8	108.4	98.9	92.6	104.5

the curves of the average changes in the two series are superimposed. The comparison demonstrates an initial increase of cell volume and of circulating blood pigment as a result of brief exercise. There is a slight initial increase in plasma volume. During more prolonged exercise the cell volume and circulating blood pigment tend to decrease while a dilution of the blood occurs by increase in plasma volume.

DISCUSSION.

The Plasma Changes.

Previous studies by other workers have shown that considerable reliance may be placed in plasma volume figures obtained by the vital red method when properly carried out. Our findings indicate that

the initial concentration of the blood during exercise is not due to a loss of fluid, since there is no decrease in plasma volume. On the contrary, as stated by Cohnheim[4] and others of his school, there is a distinct increase, especially when exercise is prolonged. As to the mechanism of this increase, Gross and Kestner[5] and Cohn[19] have offered considerable evidence to show that the fluid is taken from the muscles. They believe in a close connection between the increase in plasma volume and profuse excretion of sweat, but our results were obtained in dogs, animals which do not sweat in the ordinary sense. However, water is lost by them in considerable quantities in the process of panting.

The Alterations in the Distribution of Red Cells.

The calculation of total cell volume was made on the basis of the percentage volume of cells in the jugular vein blood, the total plasma volume being known. Hemoglobin, pigment volume, and red count are also influenced by changes in the ratio of cells and plasma. The initial increase in total cell volume shown in these experiments cannot have been due to a mere swelling of the cells, since the hemoglobin increased also. It might conceivably result either from a production of new blood corpuscles or a redistribution of those already in the body. The rapidity of the reaction definitely excludes the first possibility. Schneider[3] has called attention to the similarity of the cell changes during exercise to those taking place in the early stages of acclimatization to high altitudes, and he believed that a reserve supply of corpuscles from the abdominal vessels is thrown into the peripheral blood stream in both instances. Campbell and Hoagland[20] have found that during the process of acclimatization to high altitudes, the peripheral blood contains cells in greater concentration than the mesenteric blood. It is therefore quite possible that, under normal resting conditions, the peripheral vessels are less rich in corpuscles and contain a greater per cent of plasma than the abdominal, and that in response to the demands of exercise, a redistribution takes place.

[19] Cohn, E., *Z. Biol.*, 1920, lxx, 366.

[20] Campbell, W. A., and Hoagland, H. W., *Am. J. Med. Sc.*, 1901, cxxii, 654.

If we accept the view of Schneider,[3] one can readily explain how it was that Cohnheim and his coworkers[4] found a decrease in per cent of hemoglobin after the prolonged exertion of mountain climbing. They were at high altitudes before starting on these expeditions, and therefore the readjustments due to acclimatization had already increased as far as possible the per cent of cells in the peripheral blood. The increase in plasma volume accompanying exercise would therefore lead at once to dilution of the blood and the fall in per cent of hemoglobin which they actually observed.

The Changes in the Size of Red Corpuscles.

It is not impossible that changes in the size of red cells occur during exercise. A general swelling of the cells could readily lead to an increase in cell volume. However, the close parallelism between the curves of cell volume and pigment volume in the present work indicates that the results are not due to this cause—for were this the cause there would be disproportionate changes in pigment content and cell bulk. The red counts here recorded are too few in number to furnish reliable data as to average cell size. The literature on changes in cell size during exercise is extremely meager. Price-Jones,[21] working with dried blood smears, has concluded that the cells become larger. The present findings would, in general, tend to confirm this view, for the decrease in cell volume during prolonged exercise is not nearly so great as the decrease in pigment volume. One might account for this by a disappearance of some cells from the circulation and an increase in size of those that remain.

Evidences of Blood Destruction.

It will be noted that after prolonged exercise both total cell volume and pigment volume fall well below the maximum noted after 10 minutes of exercise. The decrease observed in percentage of hemoglobin and in the number of cells per cubic millimeter is less significant, being partly due to dilution. In view of the uncertain factor of cell distribution, it would be unsafe to conclude that blood de-

[21] Price-Jones, C., *J. Path. and Bact.*, 1919–20, xxiii, 371.

struction occurs during exercise on the basis of the present data alone, although there is no doubt that they are in harmony with such a view. More facts in the matter will be given in later papers.

SUMMARY.

The following changes have been demonstrated to take place in the blood of dogs during exercise.

1. An increase in the per cent of cells and hemoglobin in the blood of the jugular vein occurs early in the course of exercise. It probably results from a redistribution of red corpuscles, with an increase in their proportion in the peripheral blood.

2. As exercise is continued, there is a definite increase in plasma volume.

3. A coincident decrease both in the total cell volume and the pigment volume during prolonged exercise suggests that blood destruction then occurs.

EXPERIMENTAL STUDIES OF THE NASOPHARYNGEAL SECRETIONS FROM INFLUENZA PATIENTS.

IX. The Recurrence of 1922.

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Plates 46 to 49.

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INTRODUCTION.

The presence in New York City during the early months of 1922 of an acute respiratory infection that, clinically, resembled more or less the epidemic influenza of the autumn and winter of 1918–19 and the spring of 1920 afforded us an opportunity to repeat and extend the studies of the nasopharyngeal secretions from influenza patients which had resulted in the isolation of *Bacterium pneumosintes*. The reports of the earlier experiments have formed Papers I to VIII of the present series.[1-8]

As an introduction to our observations with material obtained during the 1922 recurrence of influenza, the facts already established may be passed briefly in review, since they form the basis for a comparison of the data recently obtained with the findings in similar experiments during the past $3\frac{1}{2}$ years.

[1] Olitsky, P. K., and Gates, F. L., *J. Exp. Med.*, 1921, xxxiii, 125.
[2] Olitsky, P. K., and Gates, F. L., *J. Exp. Med.*, 1921, xxxiii, 361.
[3] Olitsky, P. K., and Gates, F. L., *J. Exp. Med.*, 1921, xxxiii, 373.
[4] Olitsky, P. K., and Gates, F. L., *J. Exp. Med.*, 1921, xxxiii, 713.
[5] Olitsky, P. K., and Gates, F. L., *J. Exp. Med.*, 1921, xxxiv, 1.
[6] Olitsky, P. K., and Gates, F. L., *J. Exp. Med.*, 1922, xxxv, 1.
[7] Olitsky, P. K., and Gates, F. L., *J. Exp. Med.*, 1922, xxxv, 553.
[8] Olitsky, P. K., and Gates, F. L., *J. Exp. Med.*, 1922, xxxv, 813.

Summary of Earlier Observations.

Having in mind the possible presence in the nasopharyngeal secretions of influenza patients of an agent whose effects might be noted in animals, we injected the whole or the filtered nasopharyngeal washings intratracheally into rabbits. In eight instances the nasopharyngeal washings were obtained within 36 hours of the onset of the uncomplicated disease. The material from five of these patients during the epidemic wave of 1918–19 and from two during the recurrence of 1920 produced typical clinical and pathological effects in the experimental animals. On the other hand, the active agent was not obtained from twelve influenza patients, the onset of whose illness had occurred more than 36 hours previously, nor from fourteen persons free from influenza during the epidemic and interepidemic periods.[1]

The effects produced by the active agent in the experimentally infected animals bore a certain similarity to those observed in epidemic influenza in man. Clinically, the affection was typified by a rise in temperature within 24 to 48 hours, by conjunctivitis, and by a characteristic change in the blood picture—a rapidly developing leucopenia, due mainly to a critical drop in the mononuclear cells. Unless the train of events was interrupted by secondary infection, the rabbits returned to normal in the course of 2 or 3 days. Most of them were killed after 24 to 48 hours, however, for observation of the lung lesions and to obtain material for further passage, and for cultivation. In such instances a typical pathological condition was revealed, consisting of a diffuse hemorrhagic edema of the lungs, with emphysema, and with a peculiar interalveolar cellular exudate, and a similar hemorrhagic involvement of bronchi and trachea, with exfoliation of the necrotic epithelium.[1]

When lung tissue containing such lesions was ground and extracted with salt solution, the turbid suspension produced similar effects in other rabbits, and in this way the substance active in the process was transmitted for as many as fifteen passages, indicating the multiplication of a living agent rather than the mere passive transfer of some original material.

This living agent was found to have the following properties.

As it existed in the nasopharyngeal secretions in man, and in the lungs of affected rabbits, it passed through Berkefeld V and N candles, and the filtered material produced the same effects on the blood and in the lungs of rabbits and guinea pigs as the unfiltered material.[2]

When contained in bits of lung tissue, it withstood the action of a 50 per cent glycerol solution for periods up to 9 months.[2]

It produced effects upon the pulmonary tissues of rabbits which favored the invasion and infection of the lung with other bacteria, such as the pneumococcus, streptococcus, and *B. pfeifferi*.[3]

It led to the development, in experimentally infected animals, of a specific immunity against reinfection with similar material.[6]

Meanwhile, cultivation experiments had shown the presence in the active material of a hitherto undescribed microorganism. This microorganism, which was subsequently named *Bacterium pneumosintes* because of its injurious effect upon the lung, was first observed in November, 1918, in strictly anaerobic cultures of the filtered nasopharyngeal secretions of an influenza patient in the early hours of the disease. Subsequently the same bacterium was isolated from material originally derived from all the seven cases of influenza from which active material was transmitted to rabbits, and from three other cases not so transmitted. The immediate sources of the cultures were the filtered nasopharyngeal secretions of patients or, more frequently, the unfiltered or filtered lung tissue suspensions of affected rabbits. These rabbits had been intratracheally injected with whole or filtered nasopharyngeal secretions, whole or filtered lung suspensions of other affected animals, or the glycerolated lung tissue of such animals. The cultivation experiments were carefully controlled with similar materials from non-influenzal sources. All of the control materials gave negative results.[4]

The primary cultures of *Bacterium pneumosintes* were obtained under strictly anaerobic conditions in a medium composed of sterile human ascitic fluid and a fragment of fresh rabbit kidney. In this medium the microorganism developed slowly, producing a faint haze in the region of the kidney fragment that gradually extended to a depth of about 3 cm. by the 8th day and in the course of 2 weeks settled to the bottom of the tube, leaving a clear, faintly opalescent, supernatant fluid.

In stained films, *Bacterium pneumosintes* appeared as a minute bacilloid body of regular morphology, measuring 0.15 to 0.3 micron in the long axis. Usually solitary, the bacteria were often found in diplo form, and occasionally in short chains of three or four members. All of the strains decolorized uniformly by Gram's method. They were stained with some difficulty by the usual basic dyes.

The intratracheal injection, in rabbits and guinea pigs, of mass cultures of *Bacterium pneumosintes* in the earlier generations induced effects on the blood and lungs of these animals which could not be distinguished from those obtained with the active agent of the nasopharyngeal secretions of influenza patients. In addition to this identity of source and of pathogenic effect in animals, *Bacterium pneumosintes* exhibited the other qualities characteristic of the active agent. Thus the microorganism passed Berkefeld V and N filters even in remote generations. Protected by the lung tissue of rabbits affected by the injection of mass cultures, it withstood glycerolation for a period of 9 months. Infection with *Bacterium pneumosintes* reduced the resistance of the pulmonary tissues of rabbits to infection with other bacteria, such as Pneumococcus Type IV and *B. pfeifferi*.[4] Finally, by cross-immunity experiments, the antigenic identity of the various strains of *Bacterium pneumosintes* with each other and with the active influenzal agent was completely established.

Before methods had been found for the cultivation of *Bacterium pneumosintes* in simplified media, the opportunity was lost to examine for specific antibodies the blood serum of persons convalescent from influenza or of most of the animals

which had recovered from the experimental infection. Later, methods of cultivation were devised which did not interfere with the detection of specific antibodies.[8,9] Rabbits intravenously injected with such cultures produced antibodies which were recognized by precipitation, agglutination, complement fixation, and phagocytic tests, and animals injected with glycerolated lung tissue containing the active agent yielded serum that specifically agglutinated *Bacterium pneumosintes*. It is significant that the four available strains of *Bacterium pneumosintes*, three from 1918–19 and one from 1920, showed identical antigenic characters by serological and immunological tests.

More recent experiments on methods of cultivation[8] have shown that *Bacterium pneumosintes* can be grown on media consisting of meat infusion peptone broth, or nutrient agar, as a base, enriched with fresh animal or vegetable tissue, fresh defibrinated rabbit blood, or by the growth of other organisms. Surface colonies have been obtained on blood agar plates in the Brown anaerobic jar.[10] The plates have proved especially useful for the purification of contaminated cultures and for the demonstration of viable organisms in sparse primary growths in the ascitic fluid-rabbit kidney medium. When grown on media containing nutrient broth, and especially in the presence of dextrose, *Bacterium pneumosintes* has developed larger bacillary forms up to 1 micron in length. The identity of these microorganisms with the original strains has been proved by serological reactions and by their reversion to the minute forms on transfer to the original medium.

Animal Transmission and Cultural Studies with Recent Materials.

As a result of the experiments outlined above, we had facilities for animal transmission and cultural studies at hand during the recent outbreak of clinical influenza in January and February of the present year and were able to proceed without the loss of time that was formerly spent in an empiric search for methods.

This outbreak of influenza enabled us to examine the nasopharyngeal secretions of nine persons who had within a few hours developed symptoms characteristic of the disease. The following protocol is cited as a typical example of the cases studied.

Case 36.—Adult male. Feb. 9, 1922. Illness began at night with chilly sensations, a thin, clear secretion from the nose, fever, and pains in the joints and muscles, especially those of the back and legs. Feb. 10. Remained in bed. Temperature 38.3°C., marked coryza, intense injection of conjunctivæ, dry cough. Physical examination showed no pulmonary involvement. Leucocytes

[9] Gates, F. L., *J. Exp. Med.*, 1922, xxxv, 635.

[10] Brown, J. H., *J. Exp. Med.*, 1921, xxxiii, 677; 1922, xxxv, 467.

4,000, of which 3,920 were polymorphonuclear neutrophils, and 80 were mononuclears. On this day, 12 hours after onset, nasopharyngeal washings were obtained for examination. Feb. 11. Patient's condition improved. Feb. 12. Temperature normal. Recovery uneventful.

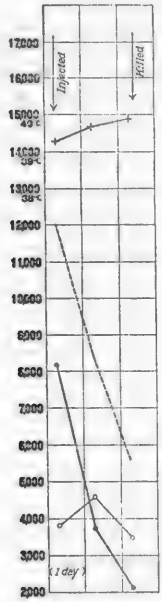
Eight other patients whose nasopharyngeal secretions were obtained from 6 to 36 hours after onset exhibited symptoms similar to those described. The washings from one of these patients stood at room temperature for 24 hours before transmission and cultivation experiments were undertaken. Both failed. Of the remaining eight specimens, successful transmission experiments were initiated with six. The seventh failed to induce the experimental disease in rabbits. The eighth specimen was not injected into animals, but after filtration through a tested Berkefeld candle it yielded a primary growth of *Bacterium pneumosintes* in an anaerobic ascitic fluid-rabbit kidney medium.

The following protocol of a typical animal transmission experiment is reported for comparison with the recorded results of the earlier experiments.[1]

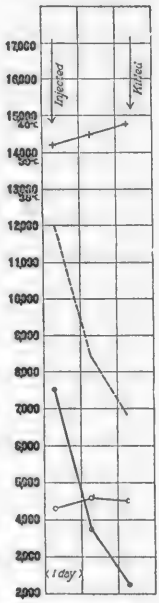
Case 28.—The unfiltered nasopharyngeal washings obtained 6 hours after onset were injected intratracheally into Rabbit A.

First Passage. Rabbit A (Text-Fig. 1).—Jan. 25, 1922. Leucocytes 12,000, of which 3,840 were polymorphonuclear cells and 8,160 were mononuclear cells. Temperature 39.2°C. Injected intratracheally with 2.5 cc. of unfiltered nasopharyngeal washings of Case 28. Jan. 26. Leucocytes 8,400, of which 3,780 were mononuclears. Temperature 39.7°C. Jan. 27. Leucocytes 5,600, of which 2,128 were mononuclears. Mild conjunctivitis. Temperature 39.9°C. Killed. All organs appeared normal except the lungs. These showed a diffuse edema and emphysema with a number of small hemorrhages scattered over the surface and on the cut sections. There was an absence of pneumonic consolidation. On microscopic examination (Fig. 1) the edema, emphysema, and the small hemorrhages were again revealed. There was a moderate degree of cellular infiltration of the interalveolar strands and about the bronchi and bronchioles, which consisted of mononuclears, polymorphonuclears containing acidophilic granules, and a few cells of the respiratory type. In addition, the interalveolar strands were distended with serum and erythrocytes. The bronchial system was also involved in a hemorrhagic and edematous process. The epithelium was necrotic in some areas and the lumen was filled with serum, leucocytes, erythrocytes, and exfoliated necrotic epithelial cells. Impression smears of the lung stained by Gram's method revealed numerous mononuclears and alveolar cells, but no bacteria. Aerobic cultures were free from growth.

In Giemsa-stained preparations of sections of the lung groups of minute organisms indistinguishable morphologically from *Bacterium pneumosintes* were noted in the bronchial exudate. Some were free and others in the different stages of phagocytosis by polymorphonuclears and monocytes (Fig. 2). In the ciliary margin of the bronchial epithelium similar clumps of these microorganisms were seen (Fig. 3).



TEXT-FIG. 1.



TEXT-FIG. 2.

+——+ Temperature.
- - - - Total leucocytic count.
•——• Mononuclear cells.
o——o Polymorphonuclear cells.

TEXT-FIG. 1. Effect on the blood count and temperature. The rise in temperature and the depression in the total white blood cell count caused by a deficiency of mononuclears are shown. First rabbit passage of the nasopharyngeal washings from a patient (Case 28) in the early stage of uncomplicated epidemic influenza (1922). Intratracheal injection.

TEXT-FIG. 2. Effect on the blood count and temperature. The rise in temperature and the depression in the total white blood cell count caused by a deficiency of mononuclears are shown. Rabbit passage of *Bacterium pneumosintes* from a culture, in the third generation, originally derived from a rabbit inoculated with the washings from another influenza patient (Case 36).

In the present series of experiments animal passage failed after the first transfer in one instance. The other transmission experiments were discontinued after the second to fourth transfers.

The effects produced by the intratracheal injection into rabbits of these specimens of unfiltered nasopharyngeal secretions from influenza patients appeared to us to be identical with those observed during the former epidemic waves. The active agent showed other familiar qualities also. It survived the action of 50 per cent glycerol for 4 months (the longest period yet tested) and passed Berkefeld V and N filters, producing characteristic lesions in rabbits injected with the filtrate.

As controls for these experiments, we have negative transmission experiments with the nasopharyngeal secretions of three persons not suffering from influenza. One of these had a mild head cold which ran a typical course; the others were normal.

Because of the identity of the active agent with *Bacterium pneumosintes* in former experiments, our endeavor was directed mainly toward the demonstration of a filterable anaerobic microorganism in the material obtained during this recurrence of influenza. Filtered nasopharyngeal secretions from each of the nine influenza patients and from ten other persons were inoculated into ascitic fluid and rabbit kidney tubes under a vaseline seal, and spread upon blood agar plates in an anaerobic jar. The lung tissues of affected rabbits were likewise cultured in the fluid medium, and sometimes smeared on the anaerobic plates. When no organisms or only a few minute and questionable bodies could be demonstrated with the microscope in fluid cultures, material from these cultures was spread on blood plates for the possible observation of colony formation.

By these methods there were obtained cultures of strictly anaerobic, filter-passing, Gram-negative organisms in material derived from four of the six influenza patients from whom an active agent had been transmitted to rabbits, from a seventh influenza patient whose nasopharyngeal washings were not injected into animals, and from four of ten other persons not suffering from influenza. A survey of these microorganisms showed that *Bacterium pneumosintes* is not the only anaerobic, filter-passing, Gram-negative organism to be found in the human respiratory tract. From one of the influenza patients and from

the four non-influenzal controls which yielded positive cultures, other bacteria, not pathogenic for rabbits and not *Bacterium pneumosintes*, were obtained. A preliminary description of these other bacteria is appended to this paper.

On the other hand, we have identified as *Bacterium pneumosintes* the cultures derived from three of the six influenza patients, enumerated above, whose nasopharyngeal secretions were pathogenic for rabbits, and from the seventh patient, whose secretions were not used in a transmission experiment. No cultures of *Bacterium pneumosintes* were obtained from the controls.

It is noteworthy that only one strain of *Bacterium pneumosintes* was obtained directly from the filtered nasal washings. The others were derived from lung tissue after rabbit passage. In this connection the relative value of these two immediate sources may be indicated. Nasopharyngeal secretions require filtration before direct cultivation in fluid media and much of the active material, mixed with mucus and epithelial debris, is retained on the filter and lost. It is probable that conditions are only occasionally favorable for the primary development of *Bacterium pneumosintes* in artificial culture. As with the pneumococcus a relatively large inoculum is usually required to initiate growth. Moreover, if the first inoculations fail the entire opportunity is lost. But when the whole nasopharyngeal washings are injected into rabbits, the larger primary inoculum is further augmented by active multiplication in the pulmonary tissues, and in those favorable instances in which contaminating bacteria are suppressed, a material rich in *Bacterium pneumosintes* in pure culture is available for artificial cultivation either directly or after filtration. Moreover, through continued rabbit passage repeated opportunities are afforded for cultivation experiments and sooner or later they usually succeed. We feel that although direct cultivation of filtered nasopharyngeal secretions may be a suitable method for the demonstration of other anaerobic filter-passing microorganisms, a negative experience in the cultivation of *Bacterium pneumosintes* without the aid of animal passage is of little significance.

Identification of New Strains of Bacterium pneumosintes.

The identification of the new strains of *Bacterium pneumosintes* was made on morphology, cultural characters, filterability, typical pathogenicity for rabbits, resistance to glycerol, reduction of resistance to secondary infection, and serological and immunological reactions.

Not all of these tests have been made with each strain, but each has been definitely identified by means of a combination of them, and all

have been agglutinated specifically by sera made with the 1918–19 strains as antigen. These experiments also served as a review and check on the characters of *Bacterium pneumosintes* as formerly observed, and confirmed our reported findings. For these reasons they may be described more or less in detail.

Morphological and Cultural Characters.—The morphology and cultural characters of the new strains are in all respects identical with those of the strains obtained in 1918–19 and 1920. Photographs of Strain 16,[4] from the 1918–19 epidemic, and Strain 36, obtained in February, 1922, may be compared in Figs. 5 and 6. Colonies of these strains on anaerobic blood agar plates are shown in Figs. 8 and 9.

Filterability.—Mention has already been made of Strain 32, which was obtained by direct cultivation of the filtered nasopharyngeal washings from Case 32. Other new strains, obtained from rabbit lung tissues, have passed Berkefeld V candles and initiated growth in culture media.

Pathogenicity.—So far all of the new strains tested (first to eighth generation) have retained their pathogenicity for rabbits. With one strain the process of intratracheal injection, followed by the characteristic infection and recovery of the organism from the lung tissue, has been repeated five times. The three other strains have been recovered in this manner twice. A typical protocol illustrates the clinical and pathological results of intratracheal injection of the strain of *Bacterium pneumosintes* derived from Case 36.

Rabbit A.—Mar. 15, 1922. Leucocytes 12,000, of which 7,680 were mononuclear cells. Temperature 39.2°C. Injected intratracheally with 3 cc. of a suspension of washed mass culture[4] of *Bacterium pneumosintes*, the third generation of Strain 36. Mar. 16. Leucocytes 8,400, of which 3,780 were mononuclear cells (Text-fig. 2). Temperature 39.5°C. Mild conjunctivitis. Mar. 17. Leucocytes 6,800, of which 2,244 were mononuclear cells. Temperature 39.8°C. Mild conjunctivitis. Killed. Observable lesions were confined to the lungs and trachea. The lungs were the site of a diffuse hemorrhagic edema and emphysema without any obvious consolidation. Microscopically, the hemorrhages, edema, and emphysema were noted as well as the typical cellular exudation and bronchial lesions similar to those found in the lungs of the rabbit injected with the nasopharyngeal secretions from an influenza patient, and described above (Fig. 7).

In Giemsa-stained preparations of the lung tissue small groups of microorganisms, indistinguishable from *Bacterium pneumosintes*, were found in the ciliary layer

of the bronchial epithelium. Aerobic cultures of the lung tissue showed no growth. Anaerobic cultures in ascitic fluid-rabbit kidney medium yielded pure growths of *Bacterium pneumosintes*.

Locus of Bacterium pneumosintes in Affected Lungs.—We have not heretofore reported direct observations on the presence of *Bacterium pneumosintes* in the lungs of infected rabbits. Frequently in the course of the earlier studies, in impression smears or sections of affected rabbit lungs, stained by Gram's method, with Löffler's methylene blue, or with methylene blue and eosin, minute but questionable bodies had been made out. In view of their inconstancy and the difficulty of demonstrating them with the stains employed, we had not been willing to accord them diagnostic significance. Recently in microscopic sections stained by Giemsa's method, minute blue- or violet-stained bodies, morphologically identical with *Bacterium pneumosintes*, have been found repeatedly in scattered groups in the affected pulmonary tissues. Their usual site is deep in the ciliary margin of bronchial epithelium (Figs. 3 and 4). Less often the bodies are found in mucous and serous exudate between the cilia and in the lumen of the bronchioles, where they are in process of phagocytosis by polymorphonuclear cells and monocytes (Fig. 2). Infrequently they have been found in the interalveolar strands, where they are usually surrounded by an inflammatory mononuclear cell reaction.

These minute and characteristic bodies, which have not been observed in normal rabbit lung tissue, have been clearly made out in various sections of the pulmonary tissues of six rabbits injected with the active agent of the nasopharyngeal secretions of influenza patients. From three of these rabbits pure cultures of *Bacterium pneumosintes* were obtained. The incidence and distribution of these bodies in the lesions caused by *Bacterium pneumosintes*, and their morphological and tinctorial similarity to this microorganism lead us to suppose that they are *Bacterium pneumosintes*.

Reduction of Resistance to Secondary Infection.—A peculiar and significant property of the strains of *Bacterium pneumosintes* obtained in 1918–19 and 1920 was their effect in reducing the resistance of the lung tissues of experimentally infected rabbits to invasion by other bacteria.[5] Some of the recently isolated strains have been tested in a similar manner and with similar results. For example, after

repeated trials had shown that 3 million pneumococci of an atypical Type II strain, injected intravenously, rapidly disappeared from the blood stream and produced no lesions in rabbits, the tested dose was used in controlled experiments such as the following.

Rabbit A.—May 16, 1922. Leucocytes 6,300, of which 3,090 were mononuclear cells. Temperature 39.2°C. Injected intratracheally with 3 cc. of a saline solution suspension of *Bacterium pneumosintes*, Strain 34, third generation in mass culture.[4] May 17. Leucocytes 6,000, of which 1,200 were mononuclear cells. Mild conjunctivitis. Temperature 39.5°C. Injected intravenously with 1 cc. of a saline solution suspension containing 3 million pneumococci, atypical Type II. May 18. Leucocytes 5,600, of which 2,520 were mononuclear cells. Conjunctivitis. Temperature 41.2°C. May 19. Leucocytes 18,800, of which 11,280 were polymorphonuclear cells and 7,520 were mononuclears. Conjunctivitis. Temperature 40.7°C. Blood taken for culture. Killed. The lower right lobe was voluminous, dark, and resistant to the knife. Section revealed a dark and congested cut surface. Fibrinopurulent material exuded from the bronchi. Microscopically, the exudate was found to consist mainly of polymorphonuclears with acidophilic granules and a few mononuclear and respiratory type cells with fibrin and serum. Impression smears of the lung stained by Gram's method showed numerous polymorphonuclear cells containing phagocytosed pneumococci. Free pneumococci seen also. The blood culture and the aerobic and anaerobic cultures of the lungs yielded pure growths of the pneumococcus. Diagnosis: pneumococcus septicemia and pneumonia.

Precisely similar results were obtained with the streptococcus and *Bacillus pfeifferi*. These experiments will be described in detail in the next paper of this series.

Serological Reactions.—The first anti-*pneumosintes* rabbit serum produced was obtained by five weekly injections of a suspension of live organisms (Strain 11) and showed an agglutination titer of 1:160 to 1:320 against the homologous strain.[7] Other sera, produced with live or killed cultures of Strains 16, 17, and 26, have shown a dilution limit of 1:16 to 1:32 for complete agglutination, even after prolonged immunization. We are inclined to believe that, aside from individual variation in rabbits, the relatively low titer of immune serum to be obtained with *Bacterium pneumosintes* is characteristic of the organism.

For diagnostic purposes this low titer of the anti-*pneumosintes* serum is not objectionable, since the reactions at dilutions of 1:8 and 1:16 are sharp and specific. We have never seen *Bacterium pneu-*

mosintes agglutinate in normal rabbit serum, even undiluted, and the bacterium shows no tendency to spontaneous flocculation in salt solution, remaining in smooth suspension for 24 to 48 hours. Dr. De Kruif, who has done some unreported experiments on the acid agglutination zone of *Bacterium pneumosintes*, informs us that it flocculates out in an unusually narrow zone, lying between pH 3.0 and 3.6, and he infers from this that *Bacterium pneumosintes* is a stable microorganism, not easily agglutinable by immune serum or other chemical or physical agents.

TABLE I.

Agglutination Tests with Old (Strains 17 and 26) and New Strains of Bacterium pneumosintes.

Strain No.	Immune Serum D.				Normal rabbit serum.				Salt solution.
	Dilutions.				Dilutions.				
	1:2	1:4	1:8	1:16	1:2	1:4	1:8	1:16	
17	+++	+++	+++	+++	−	−	−	−	−
26	+++	+++	+++	+++	−	−	−	−	−
32	+++	+++	+++	+++	−	−	−	−	−
34	+++	+++	+++	++	−	−	−	−	−
36	+++	+++	+++	+++	−	−	−	−	−
40	+++	+++	+++	+++	−	−	−	−	−

In Table I are given the results of an agglutination test with old and new strains of *Bacterium pneumosintes* against immune rabbit serum produced with one of the old strains. The serum was obtained May 6, 1920, from a rabbit intravenously injected with living cultures of Strain 11. The bacterial suspensions for agglutination were grown in *Bacillus coli* broth,[8] centrifuged, taken up in salt solution, killed by heat at 56–60°C. for 30 minutes, and standardized by opacity. The tests were set up in successive dilutions in capsules,[11] incubated at 55°C. for 1 hour, and stored in the ice box over night before macroscopic examination.

[11] Gates, F. L., *J. Am. Med. Assn.*, 1921, lxxvii, 2054.

As a further test of specificity, two of the old strains derived from Cases 16 and 17[1] and three of the new strains from Cases 32, 34, and 36 were examined for their capacity to absorb specific agglutinins.

Suspensions of the absorbing strains, of ground glass opacity, were distributed into two sets of small test-tubes in lots of 1 cc. These tubes were then centrifuged and the supernatant fluid was carefully pipetted off from the compact sediment. To each of one set of tubes was added 1 cc. of immune serum (made with Strain 16 and obtained Feb. 28, 1922) in a dilution of 1:4, the bacteria were suspended by agitation, and the tubes were incubated for 1 hour at 55°C. After centrifugation, the supernatant serum from the incubated tubes (now absorbed once) was poured on to the sediment of the corresponding culture in the second set of tubes, which were shaken and incubated for the second absorption for 1 hour at 55°C. In this way dilution of the serum was avoided. The serum samples, now twice absorbed, together with an unabsorbed control which had been incubated for 2 hours at 55°C., were then used in the agglutination test shown in Table II.

In the table the columns grouped under A show the agglutination of the various strains in the unabsorbed control serum. In Group B each absorbed serum sample was tested against its own absorbing strain. The tests show that absorption of specific agglutinins had occurred but was not complete. In Group C the serum samples, absorbed with the various strains, were tested against the homologous strain, No. 16. All of the strains had removed specific agglutinins for the homologous strain, but again, absorption was evidently not complete with the amounts of bacteria employed. Finally, in Group D the serum was (incompletely) absorbed with the homologous strain, which removed part of the agglutinins for all of the other strains.

Numerous other agglutination tests with the new strains against serum made with the old ones, and *vice versa*, have proved the antigenic identity of the new strains with the old, and among themselves. If antigenic differences or variations exist by which the strains isolated in 1918–19 and 1920 can be differentiated from those obtained in 1922, we have so far failed to discover them.

Immunological Reactions.—Because the old strains, during long cultivation, had lost their pathogenicity for rabbits, we were able to carry out cross-immunity tests in one direction only; namely, to discover whether injections of the old strains protect against subse-

TABLE II.

Absorption Tests with Old (Strains 16 and 17) and New Strains of Bacterium pneumosintes.

Group A.					Group B.				
Absorbing strains.	Strains agglutinated.	Unabsorbed control serum. Dilutions. 1:8	1:16	1:32	Absorbing strains.	Strains agglutinated.	Absorbed serum. Dilutions. 1:8	1:16	1:32
Not absorbed.	16	+++	+++	+++	16	16	++	−	−
	17	+++	+++	+++	17	17	+++	+	−
	32	+++	+++	+++	32	32	+	−	−
	34	+++	+++	+++	34	34	+	+	−
	36	+++	+++	+++	36	36	++	+	−

Group C.					Group D.				
Absorbing strains.	Strains agglutinated.	Absorbed serum. Dilutions. 1:8	1:16	1:32	Absorbing strains.	Strains agglutinated.	Absorbed serum. Dilutions. 1:8	1:16	1:32
17	16	+++	−	−	16	17	+++	++	+
32	16	+++	+	−	16	32	++	+	−
34	16	+++	+	−	16	34	+	−	−
36	16	+++	+	−	16	36	+	−	−

quent infection with the new ones. Two such experiments have been performed and have indicated this protection, as the following protocol shows.

Rabbit A.—Apr. 11, 1922. Injected intratracheally with 3.5 cc. of suspension of centrifuged sediment consisting of live *Bacterium pneumosintes*, Strains 16 and 26, derived from the 1918–19 and the 1920 waves, and grown in *B. coli* broth.[8] These cultures were at this time not pathogenic for rabbits.[4] Apr. 24. Leucocytes 12,600, of which 5,544 were mononuclear cells. Temperature 39.7°C. Bled from ear vein for agglutination test. Injected intratracheally with 3 cc. of washed sediment of live mass culture[4] of *Bacterium pneumosintes*, Strain 34, derived by rabbit passage from Case 34 of the 1922 wave, in the fourth generation. Apr. 25. Leucocytes 12,000, of which 5,760 were mononuclears. Temperature 39.5°C. Conjunctivæ normal. Apr. 26. Leucocytes 10,800, of which 5,724 were mononuclear cells. Temperature 39.5°C. Conjunctivæ normal. Killed. All organs, including lungs, normal. Aerobic and anaerobic cultures of lungs were free from growth.

Rabbit B (Normal Control).—Apr. 24, 1922. Leucocytes 9,000, of which 4,500 were mononuclears. Temperature 39.2°C. Inoculated with the recent living culture of *Bacterium pneumosintes* precisely as in the case of Rabbit A. Apr. 25. Leucocytes 4,800, of which 1,536 were mononuclear cells. Temperature 39.2°C. Conjunctivitis. Apr. 26. Leucocytes 4,600, of which 1,472 were mononuclears. Temperature 39.5°C. Conjunctivitis. Killed. Lungs showed the hemorrhagic edema, emphysema, peculiar cellular exudation, and bronchial lesions characteristic of the action of *Bacterium pneumosintes*. Aerobic cultures of lungs were free from growth; anaerobic culture yielded a pure growth of *Bacterium pneumosintes*.

We have already demonstrated[6] the presence of agglutinins against *Bacterium pneumosintes* after intratracheal injection of the active influenzal agent with which the rabbit transmission experiments were carried out. In a similar test the serum from Rabbit A, obtained 13 days after the first intratracheal inoculation with the old strains, agglutinated both the old and recent strains of *Bacterium pneumosintes*. The immunity produced by intratracheal injection, even of non-pathogenic strains of *Bacterium pneumosintes*, is therefore demonstrable by serological tests. These observations on protection and antibody formation after intratracheal inoculation agree with those of Besredka,[12] D'Aunoy,[13] and others.

SUMMARY AND CONCLUSIONS.

From the nasopharyngeal secretions of patients in the early hours of uncomplicated epidemic influenza during the recurrence in New York City in January and February, 1922, we have again obtained an active agent, pathogenic for rabbits, and have identified this active agent as *Bacterium pneumosintes*. Four new strains of this microorganism have been isolated in pure culture and identified with the 1918–19 and 1920 strains on morphological, cultural, and serological grounds. All of the significant characteristics of the old strains, including their effect upon the resistance of the lungs of rabbits to secondary invasion with other bacteria, have been noted in the new strains, which thus have served to confirm and extend our original observations.

[12] Besredka, A., *Ann. Inst. Pasteur*, 1919, xxxiii, 882; 1920, xxxiv, 361.
[13] D'Aunoy, R., *J. Infect. Dis.*, 1922, xxx, 347.

Addendum.

Reference has already been made in this paper to the discovery of other anaerobic, filter-passing, Gram-negative microorganisms, not *Bacterium pneumosintes*, in nasopharyngeal secretions from human sources. Three of the five cultures which proved not to be *Bacterium pneumosintes* were derived from the filtered nasopharyngeal secretions of supposedly normal persons. What the importance of these microorganisms may be, or whether they have any pathogenic significance we are not prepared to suggest. They indicate, however, that the cultural methods recently employed in these studies may lead to the isolation of a group, or groups, of hitherto undescribed inhabitants of the upper respiratory tract. Our observations may therefore be reported, even though fragmentary, as an indication of interesting opportunities in this field of bacteriology.

Although all the five strains of bacteria, not *Bacterium pneumosintes*, and not pathogenic for rabbits, which have been isolated, are strictly anaerobic, decolorize by Gram's method, and have passed tested Berkefeld V or N filters once or repeatedly, they differ culturally and morphologically among themselves and fall into three apparently unrelated groups.

Group I is represented by a slender vibrio which was obtained from the filtered nasopharyngeal secretions of an undoubted case of epidemic influenza from which *Bacterium pneumosintes* was not obtained.

This vibrio (Fig. 10) is approximately 0.15 to 0.2 micron in thickness, and from 0.5 to 2 microns long, with rounded ends and a smooth curve in contour which may approach a half circle. In the early generations in artificial culture media extremely minute forms were observed, but in later generations the larger forms have predominated. The culture was filtered through a tested Berkefeld V candle in the third generation. This microorganism usually takes the bacterial stain faintly and uniformly throughout. Occasionally polar staining has been observed. In fluid media, such as ascitic-kidney, dextrose broth-kidney, or *Bacillus coli* broth under a vaseline seal, it grows first in minute flakes, which soon settle out as an amorphous or flocculent sediment leaving a clear supernatant fluid. On rabbit blood plates, incubated in an anaerobic jar, it forms pin-head, dis-

crete and confluent colonies, without odor, which do not discolor or precipitate the medium (Fig. 11). The colonies are raised, convex, with an entire edge and a grayish translucency. The organism does not emulsify readily and tends to spontaneous flocculation. Serum from a rabbit immune to this organism does not agglutinate *Bacterium pneumosintes*.

Group II is represented by an extremely minute bacillus (Fig. 12) isolated from a patient with acute follicular tonsillitis. Morphologically this microorganism is not unlike *Bacterium pneumosintes*, except that it shows greater variations in length and thickness. So far we have been unable to obtain constant growths in fluid media, although the strain had survived and possibly multiplied during several weeks incubation in anaerobic blood broth. On anaerobic blood agar plates it forms extremely minute colonies, well shown in Fig. 13. In microscopic tests with the surface growth on blood agar this microorganism has failed to show any agglutination in anti-*pneumosintes* serum. It is not spontaneously agglutinable.

A third group of organisms, differing from the other two, and possibly among themselves, is found in three strains of filter passers obtained from normal throats. Morphologically these strains are also bacillary, with wide variations between the longest and the shortest forms (Fig. 14). They, too, have failed to grow consistently in fluid media, and have been maintained for many generations on anaerobic blood agar plates. The characteristic colonies of these organisms are shown in Fig. 15. A sharp cone is surrounded by a plateau with an irregular edge. These colonies are slightly brownish in color and can thus be distinguished by transmitted light. The representatives of this group flocculate in normal rabbit serum.

These fragmentary observations are reported not because of any supposed relation to *Bacterium pneumosintes*, but as an indication that other microorganisms, which must be differentiated from *Bacterium pneumosintes*, may be encountered under similar conditions. The isolation of these other bacteria has depended upon the use of anaerobic blood agar plates. The failure of all but one of them to grow in fluid media and the fact that they are not pathogenic for rabbits, under the conditions of our experiments, show why they were not encountered during the earlier periods of study, before the blood plates were used.

EXPLANATION OF PLATES.

PLATE 46.

FIG. 1. Microscopic appearance of a section of the lung of a rabbit injected intratracheally with the nasopharyngeal secretions from a patient with early influenza (Case 28). The edema, emphysema, and, in the upper portion, hemorrhage are shown. To be noted also are the distention of blood vessels with blood, the bronchial lesion (lumen filled with serum, leucocytes, and exfoliated necrotic epithelium), and the interalveolar cellular exudate. × 250.

PLATE 47.

FIG. 2. Giemsa-stained section of the lung from a rabbit injected intratracheally with the nasopharyngeal secretions from another influenza patient (Case 30). Lumen of a bronchus. Microorganisms indistinguishable from *Bacterium pneumosintes* are seen at points marked *A*, mixed with precipitate at *B*, phagocytosed by polymorphonuclear cells at *C*, and by an endothelial leucocyte, or monocyte, at *D*. The ciliary margin is indicated by *E*. × 1,000.

FIG. 3. Giemsa-stained section of the lung shown in Fig. 1. A clump of microorganisms indistinguishable from *Bacterium pneumosintes* is seen deep in the ciliary margin. Aerobic cultures of this tissue were free from growth; anaerobic cultures yielded *Bacterium pneumosintes*. × 1,000.

FIG. 4. Giemsa-stained section of the lung from a rabbit intratracheally injected with a mass culture of *Bacterium pneumosintes*, Strain 34, showing microorganisms in the ciliary layer of a bronchus. × 1,000.

FIG. 5. Stained preparation of *Bacterium pneumosintes*, Strain 16, from the 1918–19 epidemic, in fresh tissue dialysate medium.[9] Cultivated artificially for 2½ years. To be compared with Fig. 6. × 1,000.

FIG. 6. Stained preparation of *Bacterium pneumosintes*, Strain 36, from the 1922 wave, in fresh tissue dialysate medium. Cultivated artificially for 2 months. To be compared with Fig. 5. × 1,000.

PLATE 48.

FIG. 7. Microscopic appearance of a section of the lung of a rabbit injected intratracheally with a culture of *Bacterium pneumosintes*, Strain 36. To be noted are the lesions similar to those shown in Fig. 1. × 250.

PLATE 49.

FIG. 8. Colonies of *Bacterium pneumosintes*, Strain 16, from the 1918–19 wave, grown anaerobically on the surface of blood agar. × 10.

FIG. 9. To be compared with Fig. 8. Similar colonies of *Bacterium pneumosintes*, Strain 36, from the 1922 wave. × 10.

FIG. 10. Showing the morphology, after repeated subplants, of anaerobic, filter-passing, Gram-negative bacteria of Group I. From a culture in ascitic fluid-fresh tissue medium. × 1,000.

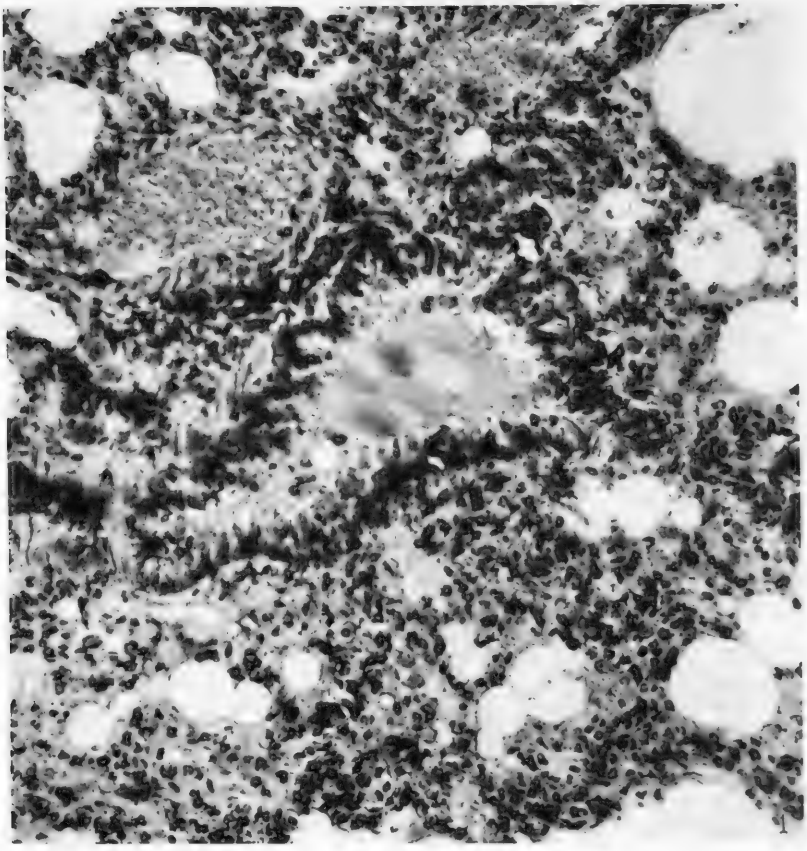
FIG. 11. Colonies of the culture shown in Fig. 10, grown anaerobically on the surface of blood agar. × 10.

FIG. 12. Morphology of anaerobic, filter-passing, Gram-negative bacteria of Group II. × 1,000.

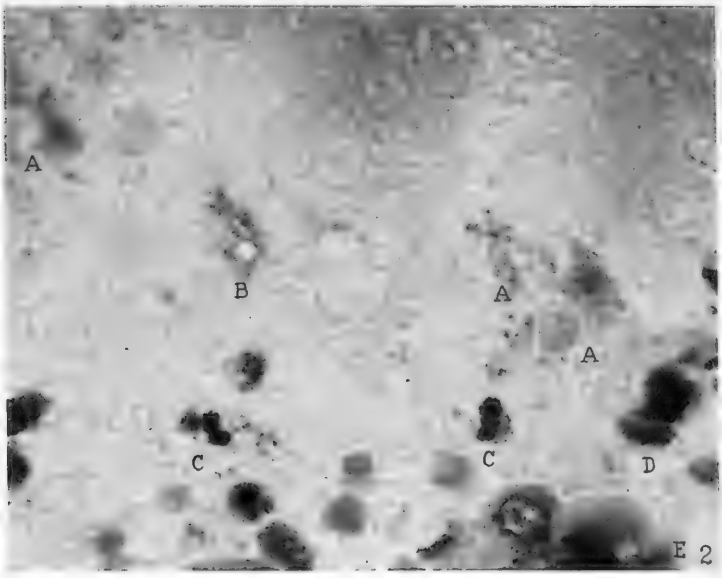
FIG. 13. Colonies of the culture shown in Fig. 12, grown anaerobically on the surface of blood agar. × 10.

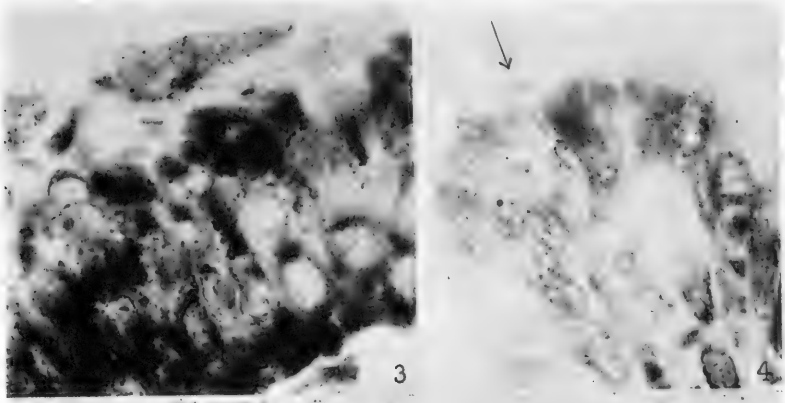
FIG. 14. Anaerobic, filter-passing, Gram-negative bacteria of Group III. × 1,000.

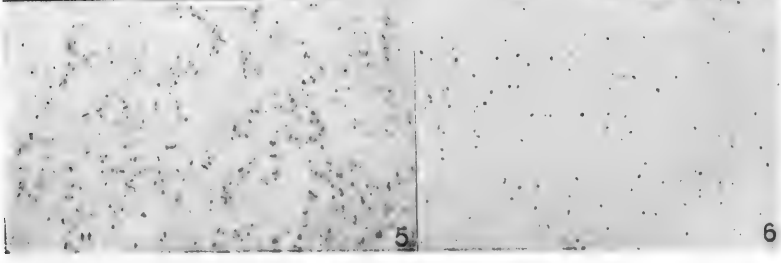
FIG. 15. Colonies of the culture shown in Fig. 14, grown anaerobically on the surface of blood agar. × 10.



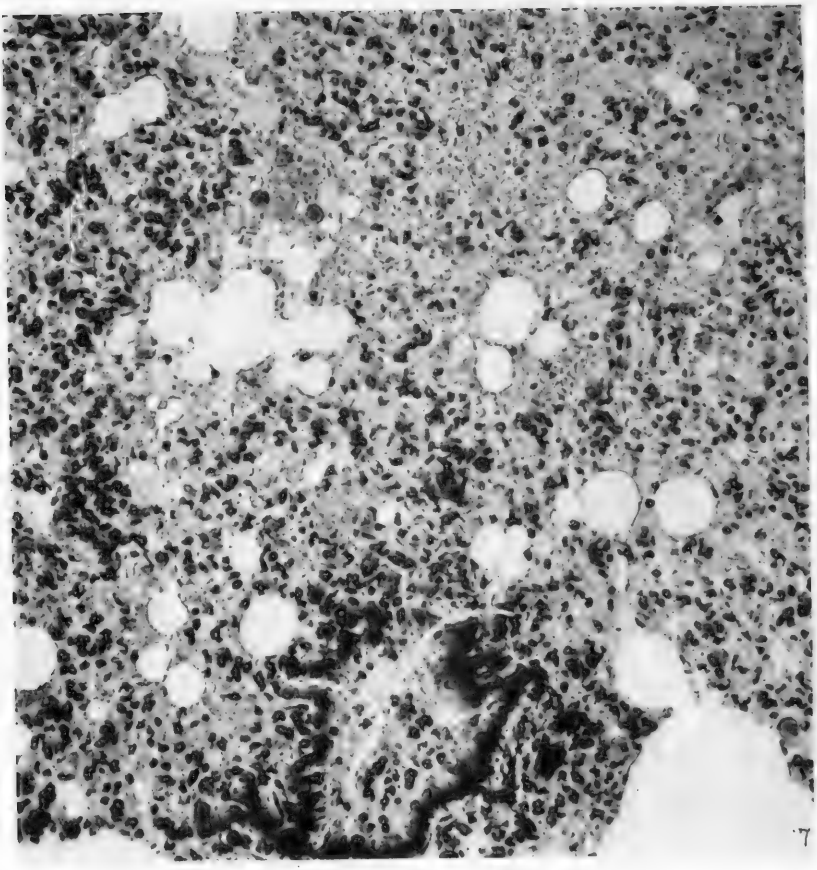
(Olitsky and Gates: Nasopharyngeal secretions from influenza. IX.)



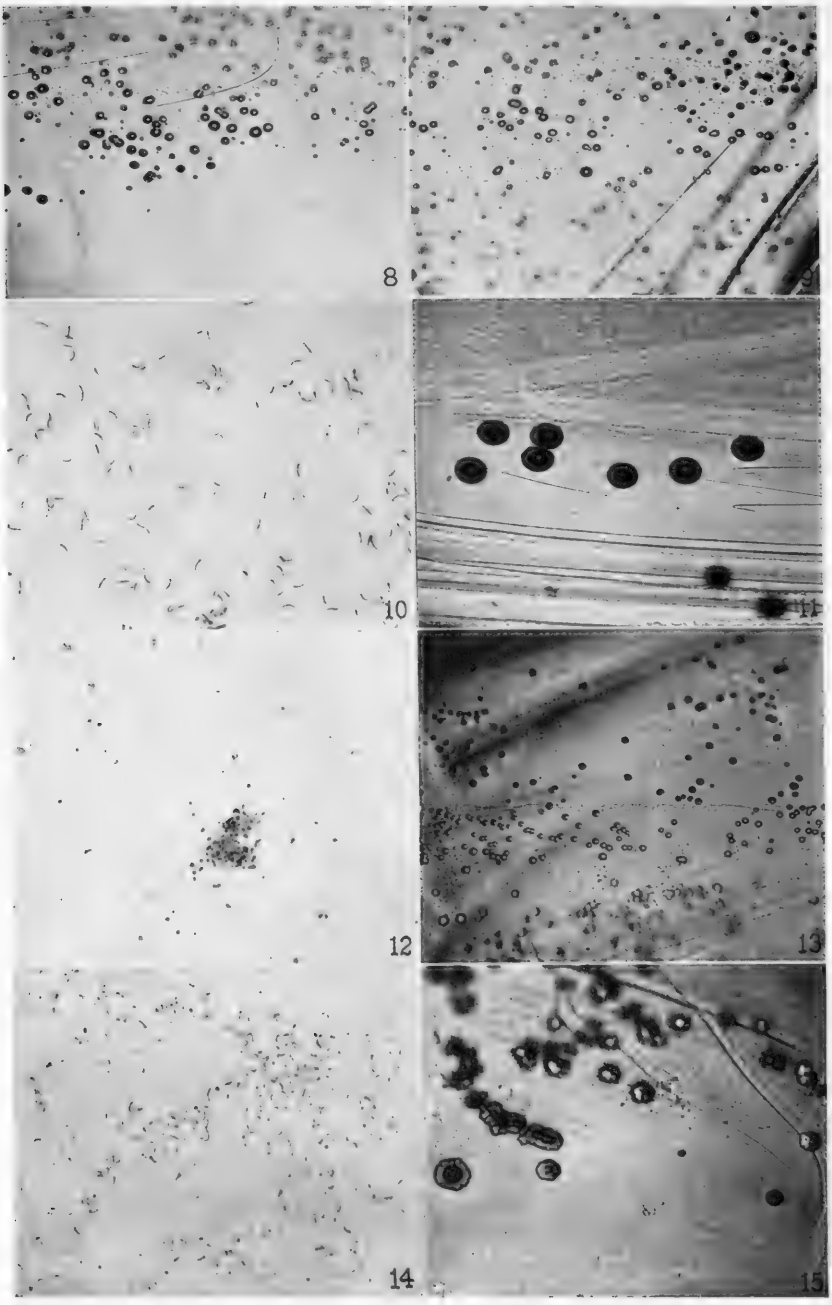




(Olitsky and Gates: Nasopharyngeal secretions from influenza. IX.)



(Olitsky and Gates: Nasopharyngeal secretions from influenza. IX.)



(Olitsky and Gates: Nasopharyngeal secretions from influenza. IX.)

DIFFERENCES BETWEEN MITOCHONDRIA AND BACTERIA.

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The suggestion that the granular and thread-like bodies, which are called mitochondria and are known to occur in almost all active cells from protozoa to man and from fungi to angiosperms, are in reality symbiotic microorganisms, would, if verified, exercise a profound influence in biology and medicine. Yet it has been repeatedly advanced in the literature.

First in 1890, Altmann[1] published a detailed account of his researches made with the help of a new method of fixation in a mixture of equal parts of 5 per cent potassium bichromate and 2 per cent osmic acid, staining in aniline fuchsin and differentiating in picric acid. By this technique the existence of mitochondria (and other granulations) was revealed in many different kinds of cells. To designate them he introduced the term bioblasts, indicating his belief that they are elementary microorganisms responsible for vital phenomena, embedded in a lifeless ground substance; but since he presented no more convincing evidence than a certain resemblance in form between the granules and bacteria, his somewhat involved theoretical considerations were not well received. They came, moreover, at a time when attention was directed toward the nucleus through studies in heredity, and the tremendous impetus of Fischer's[2] studies on the synthesis of the protein molecule lent encouragement to the view that vital phenomena might soon receive their explanation in terms of physics and chemistry. It is not surprising, therefore, that Altmann's really valuable objective findings were forgotten and investigations along this line received a decided setback.

Again in 1918 Portier[3] published detailed investigations purporting to show that mitochondria are symbiotic microorganisms, for which he proposed the term *symbiotes*. His contention was based upon a very careful review of the

[1] Altmann, R., Die Elementarorganismen und ihre Beziehungen zu den Zellen, Leipsic, 1890.

[2] Fischer, A., Fixirung, Färbung und Bau des Protoplasmas, Jena, 1899.

[3] Portier, P., Les symbiotes, Paris, 1918.

literature, but his comparison between mitochondria and bacteria is lacking in directness. For details on the mitochondrial side he has relied upon information given by others. He has not compared the morphology and staining reactions of mitochondria and bacteria occurring side by side within the same piece of tissue. His view has been supported by his colleague Bierry[4] and very severely criticized on general grounds by Regaud,[5] Guilliermond,[6] and Laguesse,[7] who, however, did not subject the problem to the test of a direct comparison.

More recently Wallin,[8] in 1922, has published observations upon the staining of bacteria with mitochondrial methods and upon the reactions of bacteria to chemical treatment which have led him independently to advance an hypothesis, like that of Portier, according to which all mitochondria are symbiotic bacteria. His is the first attempt to make a detailed microchemical study, but, like Portier,[3] the comparison which he institutes is indirect, for, obviously, the application of mitochondrial methods to bacteria means but little unless they are at the same time applied to mitochondria. From his account as it stands, it would be possible to make out a fairly strong case against the validity of his contention. For example, he notes the fact that alcohol is given as a solvent for mitochondria;[9] he also records the observation that human tubercle bacilli are not destroyed by immersion for 5 hours in 95 per cent alcohol;[10] yet he emphasizes a similarity in reaction between mitochondria and bacteria.[11] The variable resistance of bacteria to the ingredients of mitochondrial fixatives, which he has used separately and in such dilution as to produce extensive ionization, indicates further dissimilarity. One would hardly expect any traces of mitochondria to remain (as he finds the bacteria do) after treatment for 6 hours with 0.5 per cent acetic acid, with 1 per cent formalin, or with 0.5 per cent potassium bichromate.

Although the evidence presented by Altmann,[1] Portier,[3] and Wallin[8] is not convincing, certain inquiries which we have received indicate that the discussion provoked may easily lead investigators from the main problem, which is, we take it, the elaboration of new methods for the determination of the chemical constitution of mitochondria as a prerequisite to the study of their rôle in cellular physiology and pathology. Particularly is this true since the problem lies, as it were, between the sciences of cytology and bacteriology, so that but few investigators can be familiar with both sides of the question. In physiology the cell has come to be considered as a vital unit in which it is not profitable to attempt to distin-

[4] Bierry, H., *Compt. rend. Soc. biol.*, 1919, lxxxii, 131, 312.
[5] Regaud, C., *Compt. rend. Soc. biol.*, 1919, lxxxii, 244.
[6] Guilliermond, A., *Compt. rend. Soc. biol.*, 1919, lxxxii, 309.
[7] Laguesse, E., *Compt. rend. Soc. biol.*, 1919, lxxxii, 337.
[8] Wallin, I. E., *Am. J. Anat.*, 1922, xxx, 203.
[9] Wallin,[8] p. 204.
[10] Wallin,[8] p. 213.
[11] Wallin,[8] p. 221.

guish between living and non-living constituents, as Hopkins[12] clearly stated in 1913. To admit, in terms of the evidence presented, that practically all cells contain elements endowed with independent vitality would force a complete readjustment in our ideas of the organization of living matter and in the application to it of the laws of physics and chemistry. In pathology these views, if verified, would have a distinct bearing upon our conception of the significance of *Rickettsia* bodies in typhus fever, trench fever, and other conditions and would leave us in some doubt regarding the criteria to be employed in the identification of microorganisms the nature of which remains obscure.

Thus far the only reaction claimed to be specific for mitochondria is that of coloration with Janus green B (diethylsafranineazodimethylaniline chloride). Wallin[8] obtained a coloration of bacteria when this stain was employed in a concentration of 1:10,000 in physiological saline solution and uses this observation as a basis for his contention of a similarity between mitochondria and bacteria. But the comparison which he has made is not direct because he apparently relied solely upon accounts given in the literature for knowledge of the behavior of mitochondria under similar conditions. Moreover, we feel that the dye should be employed in greater dilution to bring out its specificity, as the experiments which follow will show.

EXPERIMENTAL.

We have accordingly attempted to make a direct comparison of the Janus green reactions of mitochondria in living lymphocytes and of bacteria under exactly the same conditions. Lymphocytes were chosen because, unlike fixed tissue cells, they may be brought under the microscope with very little mechanical manipulation and studied with ease in the living condition. Their reactions to Janus green have also been standardized. *Bacillus proteus*, *Streptococcus hæmolyticus*, pneumococcus, *Bacillus megatherium*, *Bacterium pneumosintes*,[13] and *Bacillus tuberculosis* (human) were selected as widely representative. They afford examples of microorganisms which are filterable and non-filterable, Gram-positive and Gram-negative, acid-resistant and -non-resistant, aerobic and anaerobic, sporulating and non-sporulating, branching and non-branching, capsulated and non-capsulated, pathogenic (for man and laboratory animals) and non-pathogenic, and which differ in morphology, so that their study may be expected to yield a comprehensive answer.

[12] Hopkins, F. G., *Nature*, 1913–14, xcii, 213.

[13] Olitsky, P. K., and Gates, F. L., *J. Exp. Med.*, 1921, xxxiii, 713.

In each case a very small amount of blood from the ear of a rabbit was placed on a slide; to this was added, with a pipette, a small drop of bacterial emulsion and a solution of Janus green B of known concentration in 0.85 per cent saline solution. The pressure of the

TABLE I.

Comparison of the Avidity with Which Mitochondria in Living Cells and Bacteria Take up Janus Green B.

Concentration of Janus green.	Mitochondria.	*B. proteus.*	*Streptococcus hæmolyticus.*	*B. megatherium.*	Pneumococcus.	*Bacterium pneumosintes.*	*B. tuberculosis.*
1:1,000	+			+			
1:2,000	+			+			
1:3,000	+			+			
1:4,000	+			+			
1:5,000	+			+			
1:6,000	+			+			
1:7,000	+			+			
1:8,000	+			+			
1:9,000	+			+			
1:10,000	+	−	+	−	−	+	+
1:20,000	+		+	−		+	−
1:30,000	+		+	−		+	
1:40,000	+		+	−		−	
1:50,000	+		+	−			
1:60,000	+		+	−			
1:70,000	+		−	−			
1:80,000	+			−			
1:90,000	+			−			
1:100,000	+			−			
1:200,000	+			−			
1:300,000	+			−			
1:400,000	+			−			
1:500,000	+			−			

The method employed was first to test the reaction of *B. megatherium* to the various concentrations of the dye. Having determined that a concentration of 1:10,000 failed to stain this microorganism, we then started at this dilution with the other bacteria, continuing until a negative reaction was reached.

cover-glass brought about a sufficient mixture of fluids. By this method the reactions of living organisms and of mitochondria within living cells may easily be compared side by side and under identical conditions. The results are indicated in Table I.

It was soon found that the mitochondria in living lymphocytes surpass all the organisms examined in their affinity for Janus green. They stain intensely in dilutions of 1:100,000 and may even be colored on the addition of one part of Janus green in half a million of salt solution. The actual dilution of Janus green in contact with the cells is considerably greater than is indicated in the table, for we must take into consideration its further dilution through admixture with the blood and the bacterial emulsion. Viewed in a test-tube a column of, for example, 1:300,000 Janus green about 1 cm. in diameter shows very little color, yet when it is applied to lymphocytes the contained mitochondria are able to concentrate it in their substance so that a mitochondrial rod having an area 1 by 0.2 μ is plainly colored when observed under the oil immersion lens with strong artificial light.

It is safe to say that none of the bacteria examined are colored on the addition of Janus green in a dilution of 1:100,000. Only two—*Streptococcus hæmolyticus* and *Bacterium pneumosintes*—were stained in a concentration of less than 1:10,000. The readings vary somewhat, depending upon the relative volumes of blood, bacterial emulsion, and of Janus green. But the study of living lymphocytes with their mitochondria intensely stained surrounded by organisms which have not taken up any of the dye leaves little room for doubt that in this respect the reactions of mitochondria and bacteria are entirely different.

For a direct comparison in fixed and stained preparations, the pancreas of the rabbit was selected as a tissue in which mitochondria are well known, through many papers[1, 14, 15] aimed at a determination of their relation to zymogen formation. The same organisms were suspended in saline solution and injected with a hypodermic syringe into the substance of small pieces of this organ (about 3 mm. in diameter), just after its removal from the body. Immediately thereafter the fragments were fixed and stained in a variety of ways. Five rabbits were used. The pancreas of the first received *Bacillus proteus* and *Streptococcus hæmolyticus*; the second, *Bacterium pneumosintes*; the third, *Bacillus megatherium* and pneumococcus; the fourth,

[14] Hoven, H., *Anat. Anz.*, 1910, xxxvii, 343.
[15] Laguesse, E., *Bibliog. Anat.*, 1911, xii, 273.

Bacillus tuberculosis and *Bacterium pneumosintes*; and the fifth was used as a normal control. By this procedure each organism was brought into immediate contact with mitochondria-laden cells with very little traumatism and without the possibility of either of them being modified through a pathological process of adjustment.

Ordinary fixatives in every-day use in cytology and in bacteriology were employed, as follows: 10 per cent formalin; Zenker's fluid with and without acetic acid; 95 per cent alcohol; Giemsa's fluid (saturated aqueous corrosive sublimate 2 parts and absolute alcohol 1 part); Regaud's fluid (3 per cent potassium bichromate 4 parts and commercial formalin 1 part); A. O. B., or Bensley's acetic-osmic-bichromate mixture (2.5 per cent potassium bichromate 16 cc., 2 per cent osmic acid 4 cc., with 2 drops of acetic acid); acetic-sublimate (saturated aqueous corrosive sublimate 25 cc. and acetic acid 1.25 cc.); and Bouin's fluid (saturated aqueous picric acid 15 cc., commercial formalin 5 cc., and acetic acid 1 cc.).

All the tissues with the exception of those fixed in 95 per cent alcohol and Bouin's fluid were treated as follows: fixed over night; rinsed in water and passed to 50 per cent alcohol at 9 a.m.; 70 per cent alcohol at 10 a.m.; 95 per cent alcohol at 11 a.m.; absolute alcohol at 12 noon; half absolute alcohol and xylol at 1 p.m.; xylol at 1.30 p. m.; 60°C. paraffin at 2.30 p.m.; and embedded at 4 p.m.

After fixation in 95 per cent alcohol tissues were passed to absolute alcohol at noon and carried on as indicated, while after Bouin's fluid they were passed to absolute alcohol at 9 a.m.; half absolute alcohol and xylol at 10 a.m.; xylol at 10.30 a.m.; paraffin at 11 a.m.; and embedded at noon, or transferred to absolute alcohol at noon and embedded as indicated above.

All sections were cut 4 μ in thickness and were stained by Giemsa's method (G), with iron-hematoxylin (FeH), and with fuchsin and methyl green (FMG). The results are given in Tables II to V.

These tables show, from the number of negative signs, that the mitochondria are in general less resistant than the bacteria. For example, they are invariably destroyed by treatment in this manner with 95 per cent alcohol and with Bouin's fluid. They are occasionally and imperfectly preserved by fixation in Zenker's with and without acetic acid, in 10 per cent formalin, in sublimate-acetic, and in Giemsa's

TABLE II.

Mitochondria, B. proteus, and Streptococcus hæmolyticus.

Tissue No.	Fixation.	Stain.	Mitochondria.	*B. proteus.*	*Streptococcus hæmolyticus.*
381	10 per cent formalin.	G	−	+	+
		FeH	+	+	+
		FMG	+	+	+
382	Zenker's fluid less acetic.	G	−	+	+
		FeH	+	+	+
		FMG	+	+	+
383	Giemsa's fluid.	G	−	+	+
		FeH	+	+	+
		FMG	−	+	+
384	95 per cent alcohol.	G	−	+	+
		FeH	−	+	+
		FMG	−	+	+
385	Regaud's fluid.	G	−	+	+
		FeH	+	+	+
		FMG	+	+	+
386	A. O. B.	G	−	+	+
		FeH	+	+	+
		FMG	+	+	+
387	Zenker's fluid with acetic.	G	−	+	+
		FeH	+	+	+
		FMG	+	+	+
388	Sublimate-acetic.	G	−	+	+
		FeH	Tr.	+	+
		FMG	−	−	−
389	Altmann's fluid.	G	−	+	+
		FeH	+	+	+
		FMG	+	+	+
390	Bouin's "	G	−	+	+
		FeH	−	+	+
		FMG	−	+	+

TABLE III.

Mitochondria and Bacterium pneumosintes.

Tissue No.	Fixation.	Stain.	Mitochondria.	*Bacterium pneumosintes.*
381	10 per cent formalin.	G	−	+
		FeH	+	+
		FMG	−	+
382	Zenker's fluid less acetic.	G	−	+
		FeH	+	+
		FMG	+	+
383	Giemsa's fluid.	G	−	+
		FeH	Tr.	+
		FMG	−	+
384	95 per cent alcohol.	G	−	+
		FeH	−	+
		FMG	−	+
385	Regaud's fluid.	G	−	+
		FeH	+	+
		FMG	+	+
386	A. O. B.	G	−	+
		FeH	+	+
		FMG	+	+
387	Zenker's fluid with acetic.	G	Tr.	+
		FeH	+	+
		FMG	+	+
388	Sublimate-acetic.	G	−	+
		FeH	−	+
		FMG	−	−
389	Altmann's fluid.	G	−	+
		FeH	+	+
		FMG	+	+
390	Bouin's "	G	−	+
		FeH	−	+
		FMG	−	+

TABLE IV.

Milochondria, B. megatherium, and Pneumococcus.

Tissue No.	Fixation.	Stain.	Mitochondria.	*B. megatherium.*	Pneumococcus.
401	10 per cent formalin.	G	−	+	+
		FeH	+	+	+
		FMG	−	+	+
399	Zenker's fluid less acetic.	G	−	+	+
		FeH	+	+	+
		FMG	−	+	+
397	Giemsa's fluid.	G	−	+	+
		FeH	Tr.	+	+
		FMG	−	+	+
403	95 per cent alcohol.	G	−	+	+
		FeH	−	+	+
		FMG	−	+	+
400	Regaud's fluid.	G	−	+	+
		FeH	+	+	+
		FMG	+	+	+
395	A. O. B.	G	−	+	+
		FeH	+	+	+
		FMG	+	+	+
398	Zenker's fluid with acetic.	G	−	+	+
		FeH	+	+	+
		FMG	+	+	+
402	Sublimate-acetic.	G	−	+	+
		FeH	−	+	+
		FMG	−	−	−
396	Altmann's fluid.	G	−	+	+
		FeH	+	+	+
		FMG	+	+	+
404	Bouin's "	G	−	+	+
		FeH	−	+	+
		FMG	−	+	Faint.

TABLE V.

Mitochondria, B. tuberculosis, and Bacterium pneumosintes.

Tissue No.	Fixation.	Stain.	Mitochondria.	*B. tuberculosis.*	*Bacterium pneumosintes.*
412	10 per cent formalin.	G	−	+	+
		FeH	+	+	+
		FMG	−	+	+
410	Zenker's fluid less acetic.	G	−	+	+
		FeH	+	+	+
		FMG	−	+	+
408	Giemsa's fluid.	G	−	+	+
		FeH	Tr.	+	+
		FMG	−	+	+
405	95 per cent alcohol.	G	−	+	+
		FeH	−	+	+
		FMG	−	+	+
411	Regaud's fluid.	G	−	+	+
		FeH	+	+	+
		FMG	+	+	+
406	A. O. B.	G	−	+	+
		FeH	+	+	+
		FMG	+	+	+
409	Zenker's fluid with acetic.	G	−	+	+
		FeH	+	+	+
		FMG	+	+	+
413	Sublimate-acetic.	G	−	+	+
		FeH	−	+	+
		FMG	−	−	−
407	Altmann's fluid.	G	−	+	+
		FeH	+	+	+
		FMG	+	+	+
414	Bouin's "	G	−	+	+
		FeH	−	+	+
		FMG	−	+	+

mixtures. This persistence after fixation in Zenker's fluid with acetic acid and in sublimate-acetic is unusual and may be due to the rapidity of dehydration. As one would expect, Regaud's fluid, A. O. B., and Altmann's fluid proved to be the best preservatives for mitochondria. The bacteria, on the other hand, resist the action of all of the ten fluids. In the preparations the distinction between the solubility of mitochondria and bacteria is often very sharp. In a single field bacteria may be seen in close contact with cells completely deprived of mitochondria (by alcohol and Bouin's fluid) although both bacteria and mitochondria have been subjected to precisely the same influences.

There is also a pronounced difference in staining reaction. Although iron-hematoxylin and fuchsin and methyl green stain both bacteria and mitochondria, Giemsa's stain, which is perhaps the best adapted for the demonstration of bacteria, colors the mitochondria little, if at all. The failure of this so called nuclear dye to color mitochondria specifically points to a difference which we must consider to be fundamental between mitochondria and bacteria. The staining of bacteria with basic dyes is usually attributed to the presence in them of chromatin-like materials containing iron. Molisch,[16] particularly, has made many contributions regarding the widespread occurrence of iron in bacteria. But Nicholson[17] has applied the Macallum[18] hematoxylin and hydrochloric acid test for iron to tissues known to contain mitochondria without obtaining any trace of coloration, which is strong evidence of the absence of masked iron in mitochondria.

In respect to their morphology also we feel that our preparations show a noticeable difference between mitochondria and bacteria. While not denying that some types of bacteria exhibit a high degree of pleomorphism and that, in searching through the body, cells may be found in which the mitochondria are much more uniform in appearance than in the pancreas, we refer to the observations of Lewis and Lewis[19] on mitochondria in tissue cultures. These investigators followed

[16] Molisch, H., Die Pflanze in ihren Beziehungen zum Eisen, Jena, 1892.
[17] Nicholson, F. M., personal communication.
[18] Macallum, A. B., *Quart. J. Micr. Sc.*, 1896, xxxviii, 175.
[19] Lewis, M. R., and Lewis, W. H., *Am. J. Anat.*, 1914–15, xvii, 339.

the changes in form of mitochondria in living cells and succeeded in demonstrating a plasticity and modifiability far greater than that possessed by any organism with which we are familiar.

DISCUSSION.

From the foregoing experiments it appears that mitochondria show microchemical and tinctorial properties differing from those of bacteria. It seems pertinent, however, to give a definition of bacteria and to indicate the points on which definite information is required before the theories advanced by Altmann, Portier, and Wallin can be considered acceptable.

According to Park and Williams:[20] "The properties of bacteria . . . which are fairly constant under uniform conditions and which have been more or less used in systems of classification, are those of spore and capsule formation, motility (flagella formation), reaction to staining reagents; relation to temperature, to oxygen and to other food material, and, finally, their relation to fermentation and disease." To which we may add the property of forming smaller or larger aggregates (colonies), the individuals of which are, however, physiologically independent (Zinsser[21]), and the development of characteristic modes of growth on suitable artificial media.

It must be admitted in all these respects that a great gulf remains between mitochondria and bacteria. In respect to mitochondria definite spores and capsules have not been noted. The mitochondrial blebs which Wallin suggests may be due to fixation bear in our judgment no resemblance to spores. They often contain easily recognizable substances like neutral fat or starch (in plants) or a clear watery chromophobe fluid, and may clearly be seen in living acinus cells of the pancreas. Motility due to flagellar action has not been observed, and certain differences in staining reaction have been noted above. The temperature, oxygen, and food requirements of mitochondria can only be expressed in terms of the requirements necessary for the vitality of the cell as a whole. It seems unsafe to conclude, as Wallin

[20] Park, W. H., and Williams, A. W., Pathogenic microörganisms, Philadelphia and New York, 7th edition, 1920, 24.

[21] Zinsser, H., A textbook of bacteriology, New York, 5th edition, 1922, 9.

has done, that because certain forms of bacteria cannot be demonstrated by staining after treatment with moist heat at 49°C. for 30 minutes they necessarily resemble mitochondria which are also destroyed by somewhat similar treatment, because many bacteria are much more resistant. No mitochondria, on the other hand, are known to resist a temperature of over 50°C. Neither is there any parallelism between mitochondria and bacteria in their relation to fermentation and to disease. In fact, their tendency is rather to decrease than to increase in diseases of infectious nature. Furthermore, there is no reason to believe that mitochondria possess the power of independent and characteristic growth apart from cells. Experiments in this laboratory[22] on the cultivation of kidney, brain, spleen, heart muscle cells, and blood in a variety of fluid and semi-solid media by aerobic and anaerobic methods have failed to reveal the multiplication of any forms at all suggestive of mitochondria.

The suggestion that mitochondria are independent microorganisms rests in our judgment upon no other evidence than a slight similarity in form of substances of about the same size.

CONCLUSION.

A direct comparison of mitochondria and bacteria in the living condition, as well as in permanent preparations, subjected to the same influences, and viewed side by side, reveals microchemical and tinctorial differences which can only be attributed to a fundamental dissimilarity in their chemical constitution.

[22] Reported in part by Olitsky (Olitsky, P. K., *J. Exp. Med.*, 1921, xxxiv, 525).

ACTION OF ANTIGEN ON FIBROBLASTS IN VITRO. II.

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It is known that a strain of fibroblasts responds to the presence of an antigen in its culture medium by becoming immunized against the action of the antigen.[1] The purpose of this paper is to report further experiments in which the influence of the antigen concentration on the process of immunization of fibroblasts *in vitro* was studied.

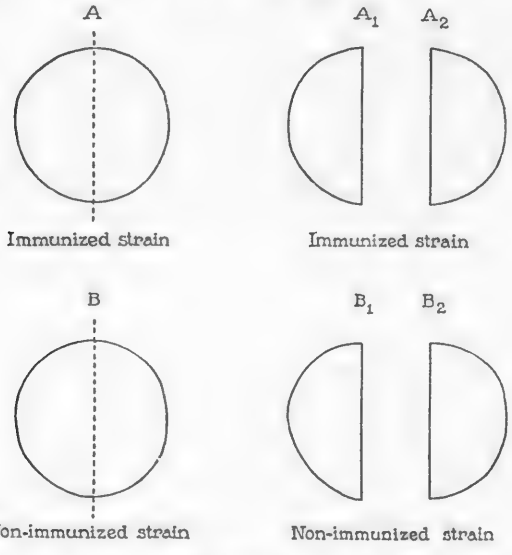
I.

Technique.

The technique was the same as described previously.[1] Dog sera and human ascitic fluid were used as antigen. The antigen was added to chick embryo juice in such a proportion that one volume of the mixture and one volume of chicken plasma would give the desired concentration. Each passage represented 48 hours, after which time the cultures were measured. The experiments were made in the following way. A fragment of tissue taken from a 10 year old strain of fibroblasts was divided into two equal parts. One was cultivated in a medium with antigen (Text-fig. 1, A); the other was cultivated in a medium without antigen (Text-fig. 1, B) and served as a control. After 48 hours incubation, the experimental and control cultures were divided into two parts. One-half of the experimental culture was transferred to a fresh medium with antigen (Text-fig. 1, A_1), and one-half of the control culture (Text-fig. 1, B_1) to a fresh medium without antigen. Both experimental and control tissues underwent many passages in media with and without antigen. The remaining halves (Text-fig. 1, A_2 and B_2) of the experimental and control cultures were placed in a medium containing the antigen under a high concen-

[1] Fischer, A., *J. Exp. Med.*, 1922, xxxv, 661.

tration, from 50 to 66 per cent, which had a marked inhibiting action on the growth of normal tissues. In this way, it was possible to ascertain the resistance gained by the immunized tissue at a given time during the experiment. If the tissue grown in a medium containing a small amount of antigen constantly did not become immunized, its rate of growth was as slow as that of the control tissue



TEXT-FIG. 1. A is a culture with a small amount of antigen. A_1 and A_2 show the same culture divided in half. A_1 is carried on in a medium containing a small amount of antigen; A_2 (subculture) is transferred to a medium containing the antigen in a high concentration. B is the control culture. B_1 is carried on as B, the control culture, and B_2 is transferred to a medium containing the antigen in a high concentration. The quotient of A_2 divided by B_2 expresses the degree of immunization of A_1.

when both were exposed to a high concentration of antigen. Therefore, the quotient of the *rate of growth of the experimental subculture* (immunized strain in the high concentration of antigen) divided by the *rate of growth of the control subculture* (non-immunized strain in the high concentration of antigen) expressed the degree of immunization. For instance, if both immunized and non-immunized strains grew at

the same rate in a high concentration of antigen, the quotient was 1, and no immunization had taken place. If the immunized strain grew more actively than the non-immunized strain, the quotient was higher than 1 and expressed the degree of immunization.

The quotient of the rate of growth in their respective media of the immunized strain divided by that of the non-immunized strain expressed the effect of the small amount of antigen in which the tissues were constantly cultivated. A quotient below 1 indicated that antigen, even in a low concentration, inhibited the growth of fibroblasts. When the quotient was 1, antigen, in the concentration used, had no effect on the fibroblasts.

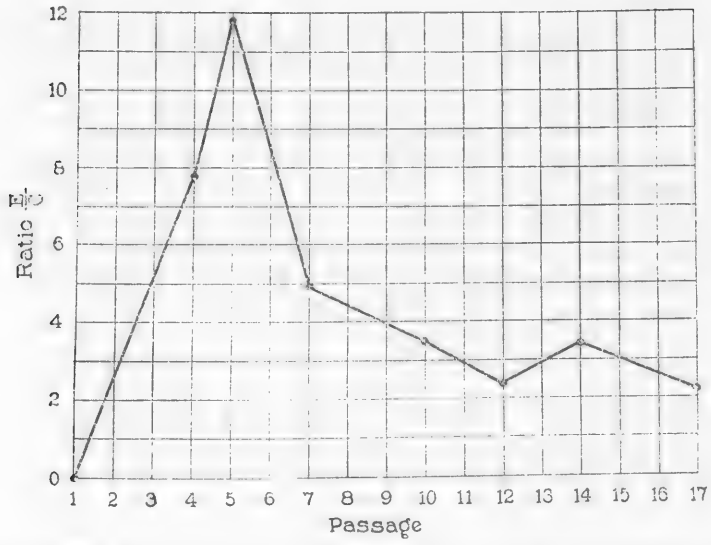
The quotient of the rates of growth of the two halves $\frac{A_1}{A_2}$ (Text-fig. 1) of the immunized strain in media containing antigen in high and low concentration expressed the effect of antigen on the immunized fibroblasts. The quotient of the rates of growth of the two halves $\frac{B_1}{B_2}$ (Text-fig. 1) of the control in media containing a high concentration and no antigen showed the effect of antigen on normal fibroblasts. These quotients were plotted in ordinates and the curves indicate that the rate of growth of the immunized strain was higher than that of the non-immunized.

II.

EXPERIMENTAL.

A. Effect of Human Ascitic Fluid as Antigen.

1. Time and Degree of Immunization of Fibroblasts under the Influence of a Small Amount of Antigen.—The total nitrogen of the human ascitic fluid used as an antigen amounted to 0.203 per cent. The culture medium of the immunized strain contained about 7 per cent ascitic fluid throughout the whole experiment. The results of an experiment which lasted for seventeen passages are recorded in Text-fig. 2. According to the diagram (Text-fig. 1), the ordinates in Text-fig. 2 represent $\frac{A_2}{B_1}$ (Table I), which is the rate of growth of both the immunized and non-immunized strains in high concentra-

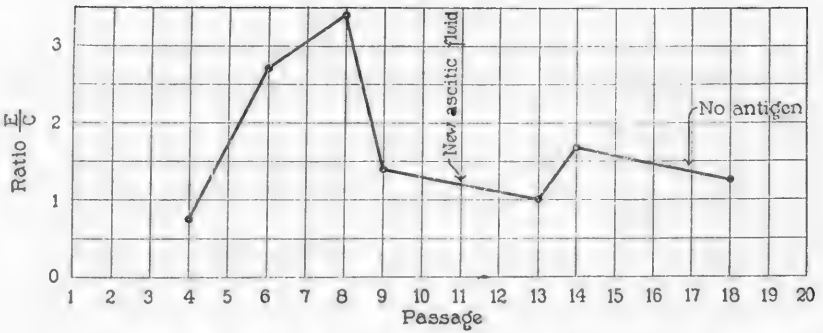


TEXT-FIG. 2. Curve showing the variations of the degree of immunization of fibroblasts through seventeen passages. Human ascitic fluid was used as antigen in a concentration of 7 per cent. The ordinates represent the quotient of the rate of growth of the immunized strain divided by that of the non-immunized strain in a high concentration of antigen, and the abscissæ the number of passages.

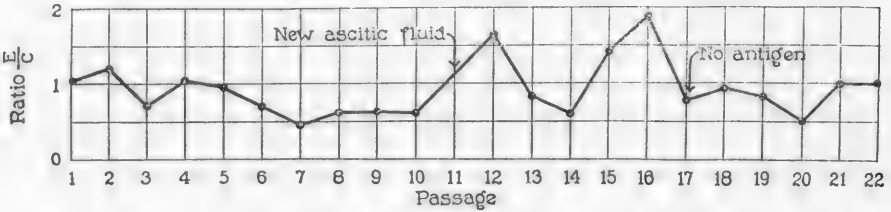
TABLE I.

Variations in the Degree of Immunization of Fibroblasts; Human Ascitic Fluid Used as Antigen in a Concentration of 7 Per Cent.

Culture No.	Passage No.	Relative increase of subcultures in 50 per cent ascitic fluid.		Ratio $\left(\frac{E}{C}\right)$ of subcultures.
		Control.	Experiment.	
847–2, 848–2	4	1.8	14.1	7.8
316–2, 317–2	5	1.1	13.0	11.8
610–2, 611–2	7	2.8	13.8	4.9
659–2, 660–2	10	4.6	16.2	3.5
686–2, 687–2	12	9.4	23.2	2.4
718–2, 719–2	14	6.0	23.0	3.4
760–2, 761–2	17	3.8	8.4	2.2



TEXT-FIG. 3. Curve showing the degree of immunization of fibroblasts through twenty-two passages. Human ascitic fluid was used as antigen in a concentration of 7 per cent. The ordinates represent the quotient of the rate of growth of the immunized strain divided by that of the non-immunized strain in a high concentration of antigen, and the abscissæ the number of passages.



TEXT-FIG. 4. Curve showing the rate of growth of the immunized strain and its control through twenty-two passages. Human ascitic fluid was used as antigen in a concentration of 7 per cent. The ordinates represent the quotient of the rate of growth of the immunized strain divided by that of its control, and the abscissæ the number of passages.

tions; namely, the degree of immunization. The maximum of resistance occurred at the fifth passage (10 to 12 days after the beginning of the experiment). Afterwards, the resistance declined slowly

TABLE II.

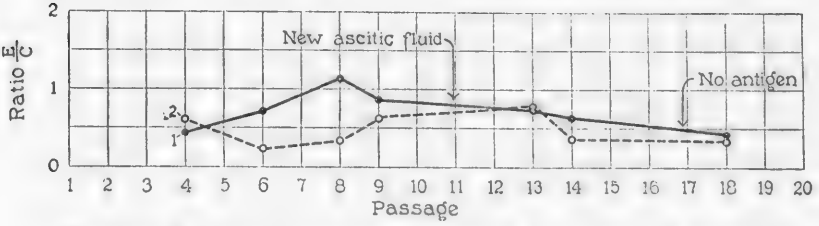
Reaction in Function of Time of Immunized and Non-Immunized Strains to a Large Amount of Antigen.

Culture No.	Passage No.	Ratio.			
		Immunized and non-immunized strains.	Non-immunized strain and its subculture.	Immunized strain and its subculture.	Subcultures of immunized and non-immunized strains.
1855–1, 1855–2	1	1.08			
1882, 1883	2	1.20			
1906, 1907	3	0.72			
1940–1, 1940–2 1941–1, 1941–2	4	1.04	0.60	0.44	0.73
1972, 1973	5	0.98			
1998–1, 1998–2 1999–1, 1999–2	6	0.70	0.28	0.70	2.70
2024, 2025	7	0.47			
2047–1, 2047–2 2048–1, 2048–2	8	0.62	0.34	1.14	3.40
2073–1, 2073–2 2074–1, 2074–2	9	0.62	0.61	0.86	1.40
2100, 2101	10	0.60			
2122, 2123	11				
2147, 2148*	12	1.63			
2170–1, 2170–2 2171–1, 2171–2	13	0.81	0.78	0.79	1.00
2199–1, 2199–2 2200–1, 2200–2	14	0.60	0.37	0.62	1.68
2217, 2218	15	1.46			
2238, 2239	16	1.90			
2266, 2267†	17	0.80			
2290–1, 2290–2 2291–1, 2291–2	18	0.94	0.32	0.40	1.25
2311, 2312	19	0.84			
2337–1, 2338	20	0.53			
2363, 2364	21	1.00			
2390, 2391	22	1.10			

* In Cultures 2147 to 2239, new ascitic fluid was used.

† In Cultures 2266 to 2391, the antigen was omitted.

and persisted still after seventeen passages (about 40 days). Another experiment of the same kind was carried through twenty-two passages. Human ascitic fluid was present continuously in a concentration of about 7 per cent in the medium of the immunized strain (Text-fig. 3 and Table II). The curve (Text-fig. 3) shows the variations of the resistance of the immunized strain. The resistance quickly reached its maximum (Text-fig. 3). Afterward, it declined, but a second maximum occurred after the thirteenth passage. The curves representing this and other experiments remind one of those obtained by Jörgensen and Madsen[2] for the amount of agglutinin produced by a goat under the influence of continuous injections of cholera bacilli.



TEXT-FIG. 5. Curve 1 shows the variations in the quotient of the rate of growth of the immunized strain in a small amount of antigen divided by the rate of growth of the same strain in a high concentration of antigen. Curve 2 shows the variations in the quotient of the rate of growth of the non-immunized strain in the ordinary medium divided by the rate of growth of the same strain in a high concentration of antigen. Human ascitic fluid was used as antigen in a concentration of 7 per cent. The ordinates represent the quotient of the rates of growth, and the abscissæ the number of passages.

2. Comparison of the Rates of Growth of Immunized and Non-Immunized Strains.—The immunized strain was cultivated exactly as was the control, with the exception of the addition of 7 per cent ascitic fluid (Text-fig. 4). If the antigen at this low concentration should have any growth-inhibiting effect, the quotient of the rates of growth of the immunized strain and its control would be below 1. As the average value of the quotient was 1, it appears that the antigen did

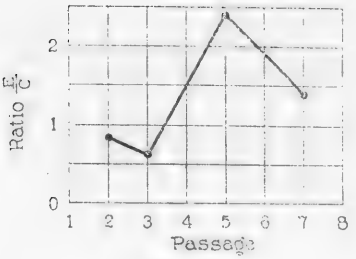
[2] Jörgensen, A., and Madsen, T., Festskrift ved Indvielsen af Statens Serum-Institut, Copenhagen, 1902, Paper 6, p. 12.

not markedly affect the activity of the strain. The variations in the quotient of the rates of growth of both subcultures of the immunized strain in low and high antigen concentrations are shown in Text-fig. 5, Curve 1. The quotients of the rates of growth of both subcultures of the non-immunized strain without antigen and in a high concentration of antigen are also shown in Text-fig. 5, Curve 2. These two curves indicate that the immunized strain grew more rapidly than the non-immunized. This has been the case in all the experiments. It seems that the presence of the antigen stimulated the growth of the fibroblasts during the entire experiment.

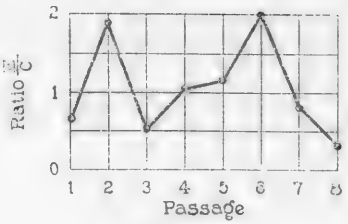
In the experiments recorded in Text-figs. 3 to 5 and Table II, new ascitic fluid was used at the eleventh passage. At the seventeenth passage, the antigen was omitted, and no change in the rate of growth occurred.

B. Effect of Dog Serum as Antigen.

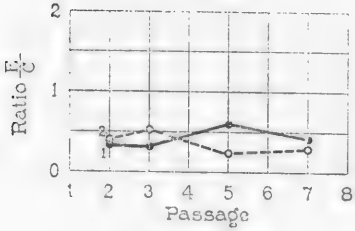
In the following experiments, dog serum was used as an antigen. When added in small quantity to the culture medium of the fibroblasts, it acted as did ascitic fluid,[1] and the fibroblasts became immunized against its action. Certain dog sera were more inhibiting for the growth of fibroblasts than others. In previous experiments,[1] the non-immunized strain of fibroblasts was often killed by a concentration of about 66 per cent, while the immunized strain remained normal. An investigation was made of the relation between the degree of immunization of a strain of fibroblasts, and the concentration of dog serum used as an antigen. Dog sera were selected which inhibited the growth of fibroblasts very markedly. The technique was similar to that previously described.[1] Fragments of a 10 year old strain of fibroblasts were immunized with antigen in different concentrations (Text-figs. 6 to 10 and Tables III to V). The antigen used was dog serum (No. 1430), in a concentration of 4 per cent. The growth index of the subcultures $\left(\frac{A_2}{B_2}\right)$, which expresses the degree of immunity, reached a maximum at the fifth passage (Text-fig. 6). The ratio between the immunized and non-immunized strains $\left(\frac{A_1}{B_1}\right)$ shows (Text-fig. 7) that, at the seventh and eighth passages, the growth index



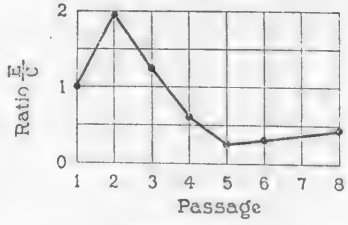
TEXT-FIG. 6.



TEXT-FIG. 7.



TEXT-FIG. 8.



TEXT-FIG. 9.

TEXT-FIG. 6. Curve showing the variations in the degree of immunization of fibroblasts through eight passages. Dog serum was used as antigen in a concentration of 4 per cent. The ordinates represent the quotient of the rate of growth of the immunized strain divided by that of the non-immunized strain in a high concentration of antigen, and the abscissæ the number of passages.

TEXT-FIG. 7. Curve showing the action of antigen on the immunized strain through eight passages. Dog serum was used as antigen in a concentration of 4 per cent. The ordinates represent the quotient of the rate of growth of the immunized strain divided by the rate of growth of its control, and the abscissæ the number of passages.

TEXT-FIG. 8. Curve 1 shows the variations in the quotient of the rate of growth of the immunized strain in a small amount of antigen divided by the rate of growth of the same strain in a high concentration. Curve 2 shows the variations in the quotient of the rate of growth of the non-immunized strain in the ordinary medium divided by the rate of growth of the same strain in a high concentration of antigen. Dog serum was used as antigen in a concentration of 4 per cent. The ordinates represent the quotient of the rates of growth, and the abscissæ the number of passages.

TEXT-FIG. 9. Curve showing the variations in the degree of immunization of fibroblasts through eight passages. Dog serum was used as antigen in a concentration of 8 per cent. The ordinates represent the quotient of the rate of growth of the immunized strain divided by the rate of growth of the non-immunized strain in a high concentration of antigen, and the abscissæ the number of passages.

TABLE III.

Immunization of Fibroblasts by Dog Serum in 4 Per Cent Concentration of Antigen.

Culture No.	Passage No.	Ratio.			
		Immunized and non-immunized strains.	Non-immunized strain and its sub-culture.	Immunized strain and its sub-culture.	Subcultures of immunized and non-immunized strains.
1180, 1181	1	0.68			
1216–1, 1216–2 1217–1, 1217–2	2	1.90	0.43	0.36	0.86
1255–1, 1255–2 1256–1, 1256–2	3	0.51	0.50	0.30	0.60
1289, 1290	4	1.03			
1326–1, 1326–2 1327–1, 1327–2	5	1.17	0.25	0.59	2.40
1361, 1362	6	2.00			
1397–1, 1397–2 1398–1, 1398–2	7	0.80	0.30	0.41	1.40
1452, 1453	8	0.30			

TABLE IV.

Immunization of Fibroblasts by Dog Serum in 8 Per Cent Concentration of Antigen.

Culture No.	Passage No.	Ratio of subcultures of immunized and non-immunized strains.
1491–2, 1492–2	2	1.95
1713–2, 1714–2	3	1.25
1489–2, 1490–2	4	0.60
1538–2, 1539–2	5	0.25
1094–2, 1095–2	6	0.30
1170–2, 1171–2	8	0.45

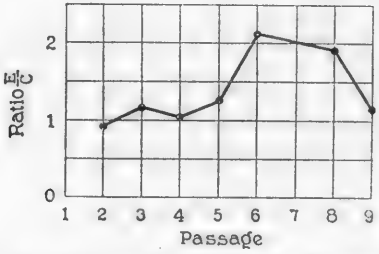
TABLE V.

Immunization of Fibroblasts by Dog Serum in 1.4 Per Cent Concentration of Antigen.

Culture No.	Passage No.	Ratio of subcultures of immunized and non-immunized strains.
1118–2, 1119–2	2	0.92
1257–2, 1258–2	3	1.14
1602–2, 1603–2	4	1.06
1670–2, 1671–2	5	1.23
1637–2, 1638–2	6	2.13
1456–2, 1457–2	8	1.90
1908–2, 1909–2	9	1.17

being under 1, the growth of the immunized strain was very much inhibited. It indicates also that after a few passages (Text-fig. 8) the immunized strain grew more actively than the non-immunized strain.

When the antigen (Dog Serum 1430) was used in a concentration of about 8 per cent, the maximum of resistance was reached at the second passage and lasted only a short time (Text-fig. 9 and Table IV). Afterwards, the resistance fell rapidly below 1. This experiment demonstrated also that, in spite of the marked inhibiting effect of an 8 per cent concentration of antigen, the strain presented a much higher resistance against dog serum than the control immediately after the second passage. When 1.4 per cent antigen was added to



TEXT-FIG. 10. Curve showing the variations in the degree of immunization of fibroblasts through nine passages. Dog serum was used as antigen in a concentration of 1.4 per cent. The ordinates represent the quotient of the rate of growth of the immunized strain divided by the rate of growth of the non-immunized strain in a high concentration of antigen, and the abscissæ the number of passages.

the culture medium, a different result was obtained (Text-fig. 10 and Table V). This low concentration of antigen restrained the growth of the immunized strain, but was less toxic for the fibroblasts than a 5 to 8 per cent concentration. The resistance reached its maximum at the sixth or eighth passage and decreased slowly afterwards.

III.

SUMMARY AND CONCLUSIONS.

The action of human ascitic fluid and of dog serum on the immunization of fibroblasts *in vitro* was investigated. The toxic effect of ascitic fluid upon fibroblasts was slight, while that of dog serum was

marked. When a small amount of antigen (1.5 to 2 per cent dog serum, or 8 per cent ascitic fluid) was present in the medium, the resistance of the fibroblasts slowly increased, reached its maximum after about 14 days, and gradually decreased. The curve expressing the phenomena closely resembles that of Jörgensen and Madsen showing the production of antibodies in an animal which received daily injections of antigen. When the antigen was present in the medium in a high concentration (from 5 to 8 per cent dog serum), the resistance reached its maximum in 4 days, and decreased rapidly.

It may be concluded that:

1. In the immunization of fibroblasts *in vitro* against a foreign protein, there is a relation between the amount of antigen, the time of the appearance of immunization, and its duration.

2. When a small amount of antigen is used, immunization slowly reaches its maximum, and slowly decreases.

3. When a large amount of antigen is used, immunization reaches its maximum in a short time, but the protection is of equally short duration.

I wish to acknowledge my indebtedness to Dr. Alexis Carrel for his permission to use the old strain of fibroblasts. I also wish to express my gratitude for the help and kindness of Dr. Carrel and his associates during my stay at The Rockefeller Institute.

SURFACE TENSION OF SERUM.

IV. Action of Temperature.

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(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

Plates 50 to 53.

(Received for publication, May 25, 1922.)

The action of heat on the surface tension of serum has been reported in previous papers,[1,2] but only at a temperature of 55°C., and for a short time. The purpose of this paper is to show the effect of prolonged heat at 55°C. on the initial value of the surface tension of pure serum, and that of temperatures of 55°, 70°, and 100°C. on the time-drop of serum solutions. In order to save space, it is assumed that the reader is familiar with the technique and terms described and used in the preceding articles.

I.

Pure Serum.

In the case of pure serum, only the initial value of the surface tension was measured, because the changes in the time-drop would be almost of the order of magnitude allowed for experimental errors. Dog serum was kept in an incubator at 55°C. for 168 hours (7 days). Measurements were made every 24 hours, according to the technique referred to above. The results are shown in Tables I to IV and Text-figs. 1 to 3.

These figures show plainly the continuous decrease of the value of the surface tension when pure serum is heated continuously. The smoothness of the curves indicates that, in most cases, the modifications undergone by the serum between the 1st and 24th hours, and between the 24th and 48th hours, for instance, are quite similar.

[1] du Noüy, P. L., *J. Exp. Med.*, 1922, xxxv, 575, 707.

[2] du Noüy, P. L., *J. Exp. Med.*, 1922, xxxvi, 115.

Consequently, the destruction of complement which takes place in the first 2 hours does not manifest itself by an important change in the value of the surface tension, or else the curves would show some kind of a break, a maximum or a minimum; moreover, if the change is

TABLE I.

Dog Serum, No. 976 (Text-Fig. 1).
Temperature 23°C.

Time, *hrs*	0	24	48	72	96	120	130
Surface tension, *dynes*	57.0	56.5	56.0	55.0	54.0	53.0	Coagulated.

TABLE II.

Dog Serum, No. 984 (Text-Fig. 1).
Temperature 23°C.

Time, *hrs*	0	24	48	72	96	120	130
Surface tension, *dynes*	56.0	55.0	53.0	51.9	52.5	50.0	Coagulated.

TABLE III.

Dog Serum, No. 998 (Text-Fig. 2).
Temperature 23°C.

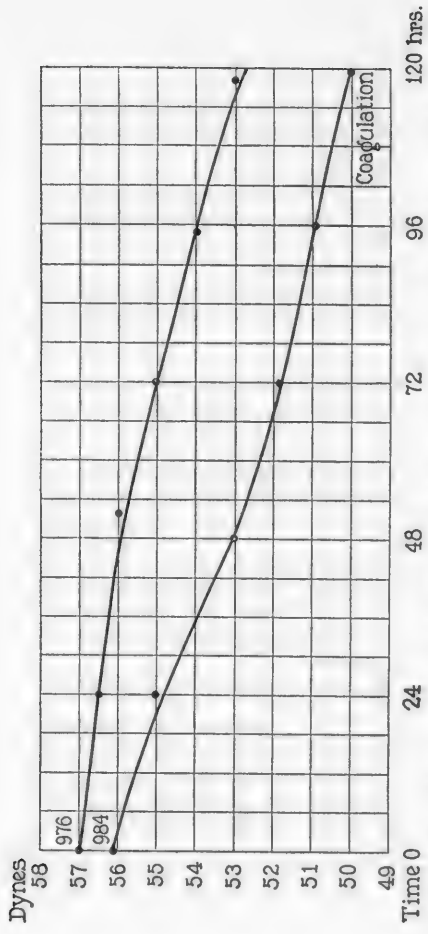
Time, *hrs*	0	24	48	72	96
Surface tension, *dynes*	57.0	57.0	56.0	55.0	54.1

TABLE IV.

Dog Serum, No. 987 (Text-Fig. 3).
Temperature 23°C.

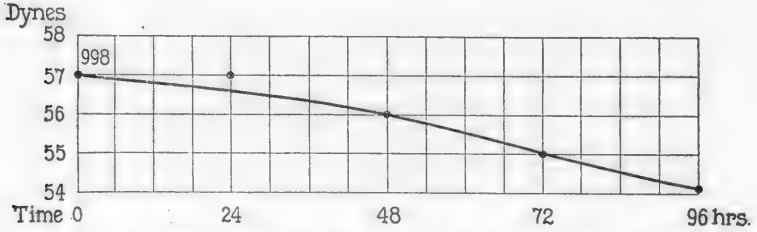
Time, *hrs*	0	24	48	72	96	120	130	168
Surface tension, *dynes*	57.5	54.5	52.3	51.3	50.8	50.5	50.0	49.2

connected in any way with the decrease in surface tension, it is so small in 2 hours that it remains within the range of experimental error. At the end of 120 hours, the reaction is in general almost as marked as at the beginning, the slope of the curve showing no constant tendency to change.



TEXT-FIG. 1. Action of heat at 55–56°C. on the surface tension of serum, in function of the time.

As the perturbations due to the destruction of complement may be of such order of magnitude that the presence of highly concentrated colloids would hinder their effect on surface tension, if there is any, experiments were carried out with solutions of serum.



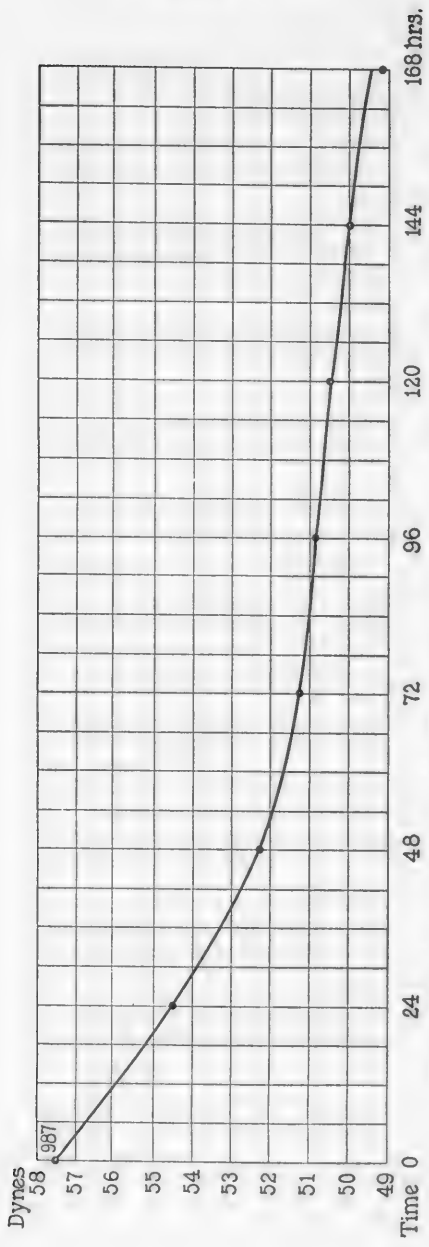
TEXT-FIG. 2. Action of heat at 55–56°C. on the surface tension of serum, in function of the time.

II.

Solutions of Serum.

All solutions were made from the same serum, with 0.9 per cent NaCl solution, especially prepared in clean vessels, the surface tension of which was 76.0 dynes ± 0.2 at 23°C. The solutions were stirred mechanically by an electric motor fitted with a small, propeller-shaped, glass stirrer. The liquid was removed from the container while the stirring was going on and poured into clean test-tubes.

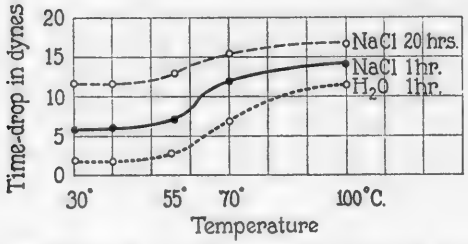
The heating was done in the following way. In most experiments six dilutions were prepared, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}. About 6 cc. of each dilution were poured into four test-tubes which were prepared at the same time. One was kept at room temperature, one at 55°C. for 2 hours, one at 70°C. for 1 hour, and one at 100°C. for 5 minutes. In order that the same time should elapse between the preparation of the solutions and the measurements, the measurements were begun only 1 hour after the tube at 55°C. had been removed from the incubator, so that approximately 3 hours elapsed between the pouring into the test-tubes and the measurements. Before the liquid was poured into the watch-glasses, it was carefully stirred. Then after 2 hours, another reading was taken. Hence the time required for one set of measurements, at one concentration, was 5 hours. In this case, the time-drop was studied because the initial values, on ac-



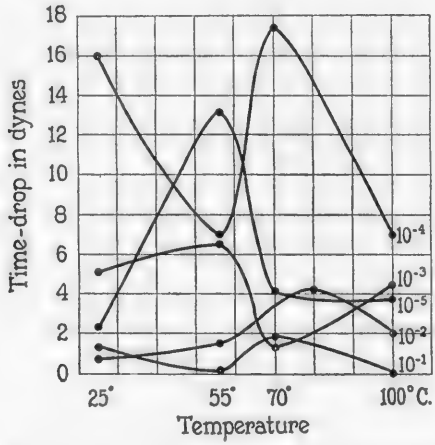
TEXT-FIG. 3. Action of heat at 55–56°C. on the surface tension of serum, in function of the time.

count of the dilution, do not show any marked differences, except when heated at 100°C.

In order to save space, and because the accuracy of the figures does not have any bearing on the general results, charts only will be

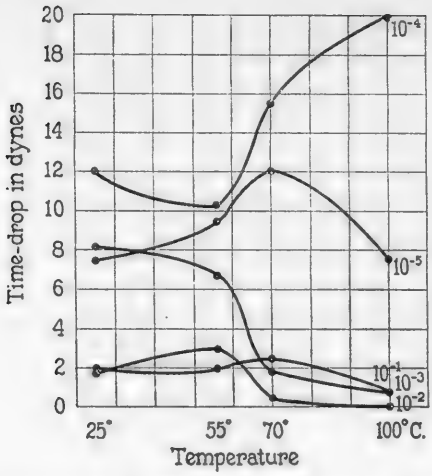


TEXT-FIG. 4. Time-drop of serum solution, concentration 10^{-4}, in saline solution and distilled water (Serum 119).

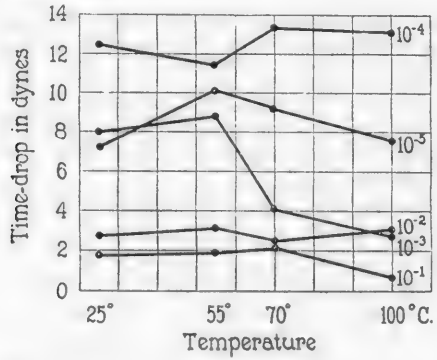


TEXT-FIG. 5. Time-drop in 2 hours, in function of the temperature, at different concentrations (Serum 1696).

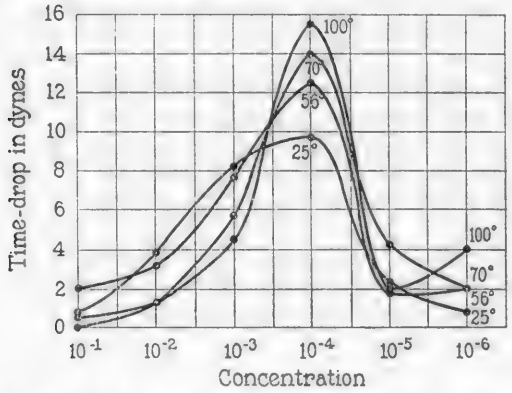
given. A curve expressing the time-drop in 1 and in 20 hours at one concentration is given in Text-fig. 4, with the curve obtained by diluting the serum in distilled water. A fair idea of how puzzling the results seem to be at first glance is given in Text-figs. 5 to 10. No regularity whatsoever is to be found. Unless an hypothesis is made, it is im-



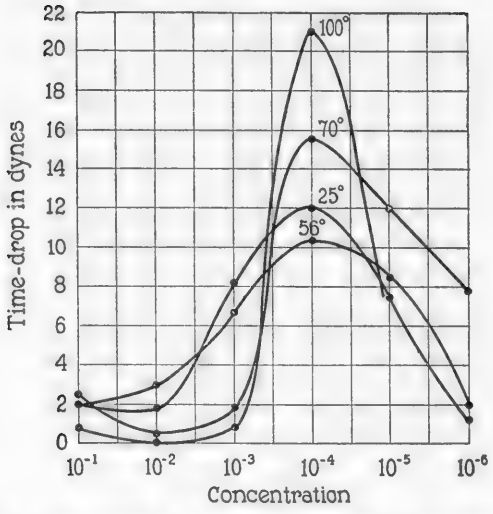
TEXT-FIG. 6. Time-drop in 2 hours, in function of the temperature, at different concentrations (Serum 1711).



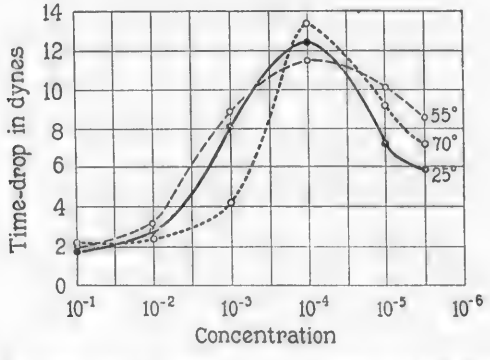
TEXT-FIG. 7. Time-drop in 2 hours, in function of the temperature, at different concentrations. Mean values of ten experiments.



TEXT-FIG. 8. Time-drop in 2 hours, in function of the concentration, at different temperatures (Serum 1802).



TEXT-FIG. 9. Time-drop in 2 hours, in function of the concentration, at different temperatures (Serum 1711).



TEXT-FIG. 10. Time-drop in 2 hours, in function of the concentration, at different temperatures. Mean values of ten experiments.

possible to interpret these results. The mean values of ten of the charts, in other words the algebraic sums of 480 measurements, are represented by Text-figs. 7 and 10.

III.

DISCUSSION.

The interpretation of these results is difficult, and it is not at all certain that it will be successful. However, we shall endeavor to give a possible explanation of the curves, which seem, at first sight, contradictory and devoid of meaning.

One is struck by the fact that the most important changes in the time-drop occur at the temperatures 55° and 70°C. (Text-figs. 5 to 7). These temperatures seem to be critical for the serum, and the fact should have attracted attention even had it not been known that they are also critical from the physiological standpoint. On the other hand, the curves representing the behavior of solutions 10^{-1} and 10^{-2} are parallel in general, and do not present the sharp jumps and drops shown by solutions 10^{-3}, 10^{-4}, and 10^{-5}. Finally, it will be noticed that the dilutions 10^{-3} and 10^{-4} generally act in an opposite way.

The first idea which naturally arises is that the surface equilibrium is more stable on either side of the concentration 10^{-4}, that is 10^{-3} and 10^{-5}, so that around 10^{-4} it reaches some sort of peak instability, and the values may rise or drop suddenly, according to the laws of hazard; namely, for such reasons as actually escape our control and knowledge. These causes, although very slight, may produce considerable changes in either direction simply because, the equilibrium being unstable, the phenomenon has just as much tendency to occur one way as the other.

Table V shows, perhaps more clearly than the charts, that the surface equilibrium at the concentration 10^{-4} seems perfectly unstable, while it is more or less determined in a given direction at all other concentrations. Here, the direction only of the time-drop has been taken into account, whether it increased or decreased, at the critical temperatures. The + sign expresses symbolically that the time-drop rose, the − sign that it decreased. As the values were taken from ten experiments, the coefficients were added to show the percentage of the occurrence of the rise or the drop; and it was admitted that one − sign checks a + sign, so that the figures actually express the number of rises or falls in excess. For example, out of the 10 experiments at a dilution 10^{-1} between 25° and 55°C., 2 showed a decrease and

8 showed an increase of the time-drop. The 2 decreases will be given the − sign, and the 8 increases the + sign. But 2 − counteracts 2 +, so that we have only 6 + left. The next entry shows that we had 9 + and 1 −, the result being 8 +. In other words, the figures could be called equilibrium coefficients, 10 being the maximum characterizing perfect equilibrium, and 0 (5 + and 5 −) corresponding to perfect instability.

Table V shows that there is a decided maximum of instability at the concentration 10^{-4}, and a much greater instability on the side of higher dilutions than on the side of lower dilutions. As a matter of fact, the presence of the 3 zeros in the 10^{-4} line may be considered as an accident, on account of the small number of experiments. But

TABLE V.

Increase and Decrease of the Time-Drop of Serum Solutions in 2 Hours When Heated at 55°, 70°, and 100°C.

Concentration.	Between 25° and 55°C.	Between 25° and 70°C.	Between 55° and 70°C.	Between 70° and 100°C.
10^{-1}	6+	10+	8+	10−
10^{-2}	8+	2−	8−	0
10^{-3}	6+	10−	10−	2−
10^{-4}	0	0	0	2+
10^{-5}	2+	2+	0	2−
10^{-6}	2−	2−	10−	2+

the results would have been just as convincing had such coefficients as 2 been found; only 100 experiments would be expected to give a real expression of the state of instability. However, there is no doubt that there is a striking difference, which could only be enhanced by a larger number of data, between the upper part of the table and the lower.

This confirms our preceding hypothesis as to the existence of a monomolecular layer at a concentration around 10^{-4}, geometrically arranged so that the sum of all individual molecular attractions in this layer is minimum, or very nearly so. Hence, any minute change, either in the arrangement of the molecules or else in their field of force, will manifest itself by a relatively considerable rise or decrease in the time-drop, owing to the state of unstable equilibrium of the

film. Therefore, it is in the vicinity of this concentration that the most sudden jumps of the surface tension are to be expected (Text-figs. 8 and 9).

From these conclusions, one fact may be remembered; namely, that if certain physiological modifications of the serum are to have a bearing on surface tension or its changes in function of the time, the possibility of detecting them will be greater at the dilution which enables one to bring forth the slightest alterations in the fields of forces of the molecules or group molecules, that is around 1:10,000.

IV.

Crystallization.

Figs. 1 to 4 show the alterations brought about by heating the solution of serum at 1:10, in the crystallization of sodium chloride. However, the changes are not always identical, especially between 25° and 56°C., and all sera do not allow the sodium chloride to crystallize in the same way. The pictures reproduced here are, nevertheless, frequently observed, and other configurations may be considered as more or less exceptional. The reader is referred to the preceding paper[2] for another manifestation of the action of heat on solutions of serum.

V.

CONCLUSIONS.

1. The surface tension of pure serum, heated at 55°C., decreases progressively and regularly until the serum coagulates. A drop of 8.2 dynes was observed in 168 hours. The mean drop was 5.7 dynes in 120 hours and 4 dynes in 96 hours.

2. The initial surface tension of solutions of serum at the concentrations 10^{-1} up to 10^{-6} is practically not affected by heat, but the time-drop in 2 hours is modified.

3. Each serum seems to react in its own particular way as far as the time-drop is concerned. However, there is a general tendency for the solution to show an increase of time-drop at the concentrations 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-5}, and a decrease at 10^{-4}, when heated at 55°C.; a decrease of the time-drop at the concentrations 10^{-1}, 10^{-2}, and 10^{-3}, and an increase at 10^{-4} when heated at 100°C.

4. Nevertheless, only the changes observed below 10^{-3} are constant in sign, within 75 or 80 per cent of the cases. The concentration 10^{-4} seems to correspond to a state of greater instability. This confirms the hypothesis of the existence of a monomolecular layer at that concentration, which was assumed on the basis of the existence of a maximum drop at 10^{-4}. Should this be true, and provided the principle of Gibbs could be transformed so as to be applicable to mixed solutions of colloids and crystalloids, an idea of the size, or at least of one of the dimensions of the molecules or group molecules composing the serum could probably be obtained.

5. 1 drop of the solutions at 1:10 evaporated on glass and examined under the microscope shows the marked differences brought about by heating at different temperatures.

A great number of the measurements referred to in this paper were made by my technical assistant, Mr. John Zwick, to whom I wish to express my indebtedness.

EXPLANATION OF PLATES.

PLATE 50.

FIG. 1. Photomicrograph of 1 drop of serum diluted in 1:10 saline solution. Kept at room temperature. × 20.

PLATE 51.

FIG. 2. Photomicrograph of 1 drop of serum diluted in 1:10 saline solution. Kept at 55°C. for 2 hours. × 20.

PLATE 52.

FIG. 3. Photomicrograph of 1 drop of serum diluted in 1:10 saline solution. Kept at 70°C. for 1 hour. × 20.

PLATE 53.

FIG. 4. Photomicrograph of 1 drop of serum diluted in 1:10 saline solution. Kept at 100°C. for 5 minutes. × 20.

FIG. 1.

FIG. 2.

(du Noüy: Surface tension of serum. IV.)

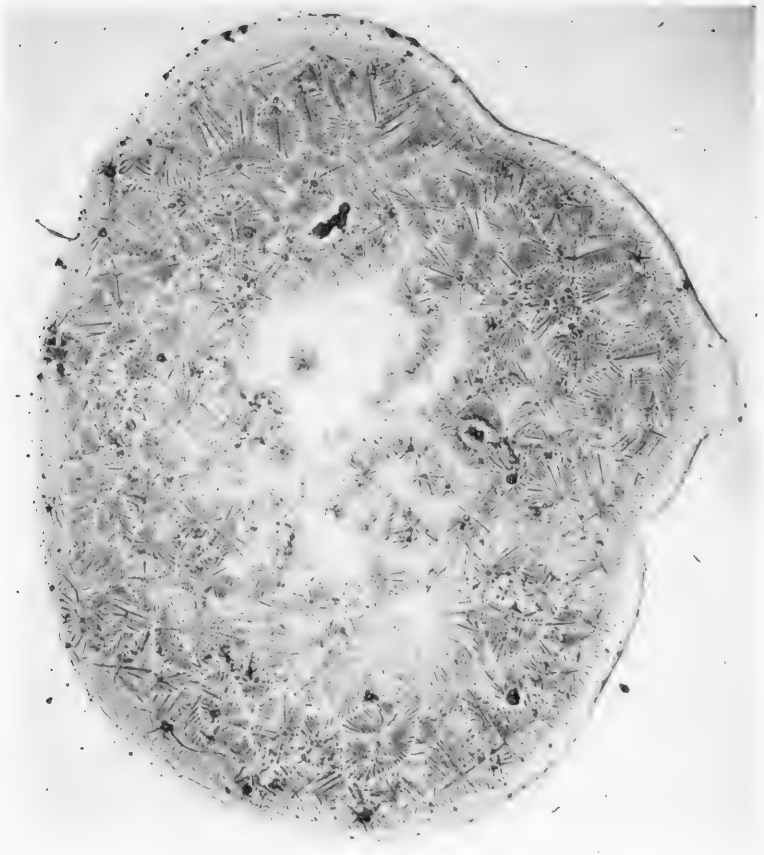


FIG. 3.

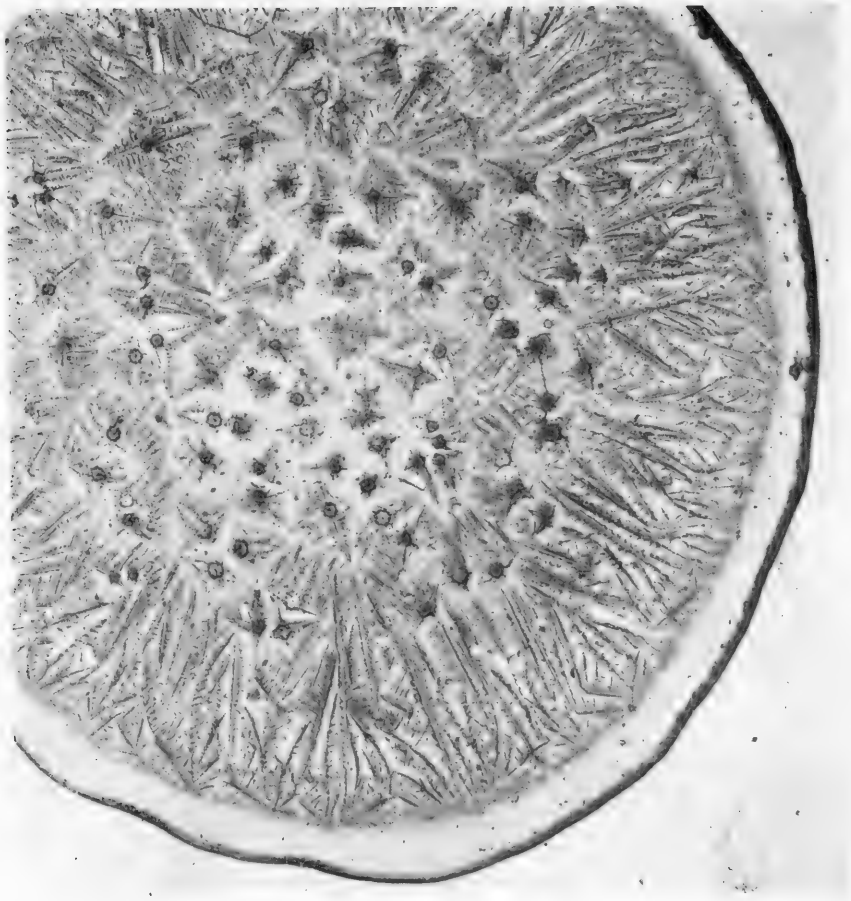


FIG. 4.

(du Noüy: Surface tension of serum. IV.)

INVESTIGATIONS ON THE OXYGEN CONTENT OF CUTANEOUS BLOOD (SO CALLED CAPILLARY BLOOD).

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INTRODUCTION.

It is generally presumed that cutaneous blood (so called capillary blood) is similar to arterial blood, particularly if the skin is made hyperemic by rubbing, or application of heat, etc., before the incision is made (Naegeli (1)). That cutaneous blood, at any rate so far as blood sugar is concerned, differs from the venous blood, has been shown by Hagedorn (2), who also uses the hypothesis that the capillary blood is similar to arterial blood in that respect. In the following experiments we have taken up the question of the composition of the cutaneous blood and especially its relation to the arterial blood. We have used the oxygen content as an indicator of the nature of the blood, first, because the amount of oxygen in the blood generally changes during the blood flow through the capillaries, and second, because oxygen unlike all other substances in the blood has the property[1] that its maximum amount in the blood can be determined outside the body, this being equal to the sum of the total oxygen capacity of the hemoglobin and the amount dissolved in the plasma. If no appreciable change takes place in the concentration of the hemoglobin when the blood flows from the arteries to the cutaneous incision, the oxygen content of the arterial blood must lie between the total oxygen capacity and the content of the cutaneous blood. It is *a priori* probable that this range is small and that for many purposes cutaneous blood could be substituted for blood obtained by

[1] This property is a consequence of the form of the oxyhemoglobin dissociation curve.

TABLE I.

Determinations of the Oxygen Content of Cutaneous Blood and of the Total Oxygen-Combining Power of the Blood under Different Physiological and Pathological Conditions.

Experiment No.	Subject No.	Nature of blood.	Hemoglobin (Haldane's scale).	Total oxygen-combining power of blood.	Oxygen content of blood sample.	Oxygen unsaturation.	Oxygen saturation.	Freezing of finger-tip.	Remarks.
			per cent	*vol. per cent*	*vol. per cent*	*vol. per cent*	*per cent*		
1	1	Arterial.	108	(20.0)*	19.49	0.51	97.5		Normal resting individual.
		Cutaneous.	108	(20.0)	19.32	0.68	96.6	−	
		Venous.	108	(20.0)	15.01	4.99	75.0		
2	2	Cutaneous.	(102)	18.79	18.42	0.37	98.0	+	" " " Blood drawn immediately after incision.
		Venous.	(102)	(18.79)	12.36	6.43	65.8		
3	2	Cutaneous.	101	(18.63)	17.54	1.09	94.3	−	Normal individual immediately after vigorous exercise.
		Venous.	(102)	18.94	13.68	5.26	72.3		
4	2	Cutaneous.	(102)	22.18	21.78	0.40	98.2	+	" " " " " " Incision made before exercise.
5	3	"	76	(14.05)	13.09	0.96	93.2	−	Nurse, age 24, suffering from exudative pleurisy. No cyanosis. Left side entirely incapacitated.
		Venous.	(78)	14.31	10.42	3.91	72.7		
6	4	Cutaneous.	(121)	22.49	22.08	0.41	98.2	+	Woman, age 32, suffering from mitral stenosis. Relative insufficiency. A few râles, both lungs. Two first samples taken after rest; third after slow walking upstairs. For cyanosis, see the text.
		Venous (1).	(110)	20.39	14.12	6.27	69.3		
		" (2).	(110)	(20.39)	5.99	14.40	29.4		

7	5	Cutaneous (1).	(121)	(22.36)	21.90	0.46	97.9	+	Patient, age 40, with emphysema, bronchial asthma, and chronic bronchitis. No attack of asthma in last 24 hrs. Several rhonchi, a few coarse râles heard at back of both lungs. No fine râles. Heart normal. The two first samples taken after rest and immediately after incision; last two samples taken after patient had walked twice up and down one flight of stairs. No cyanosis present.
		Venous (1).	(121)	22.36	18.52	3.84	82.9		
		Cutaneous (2).	(126)	23.38	20.82	2.56	89.1	−	
		Venous (2).	(126)	(23.38)	19.47	3.91	83.3		
8	1	Cutaneous (1).	(101)	18.71	11.81	6.90	63.1	+	Normal individual at rest. Two first samples (cutaneous blood 1 and venous blood) taken when subject inspired air with very low oxygen tension (42.77 mm.). Very heavy generalized cyanosis was observed at that time. Blood drawn immediately after incision. The third sample (cutaneous blood 2) was taken shortly after the first and from the same incision, but at a period during which the subject inspired normal air. No cyanosis was present at that time.
		Venous.	(117)	21.64	10.50	10.50	51.5		
		Cutaneous (2).	(100)	18.52	17.62	0.90	95.14	−	

*The figures in parentheses are the calculated figures.

arterial puncture. The object of these experiments was to show whether this is actually the case.

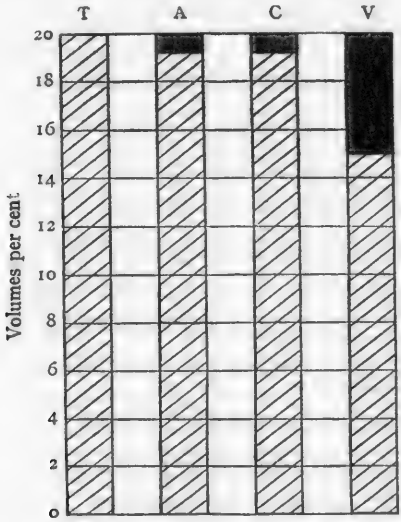
Technique.

1. Drawing the Blood.—Arterial puncture, which was made in only one instance (Experiment 1), was performed according to the technique of Hürter (3), Stadie (4), and Harrop (5). Venous puncture was done according to the procedure devised by one of us (6). For collecting the cutaneous blood the following method was devised. With a very sharp knife, from one to three incisions were made in the finger-tip, in some instances without, and in some instances with previous freezing with ethyl chloride (Table I). The incisions were from 1 to 1.5 cm. long, and approximately 3 mm. deep and were made lengthwise on the finger-tip. After the incision was made and the first drop of blood taken away, the subject himself compressed the wound with the thumb of the same hand and the two fingers were put down under oil in a jar. Pressure with the thumb was then discontinued, the blood thus being allowed to run freely under the layer of paraffin. A small amount of pulverized potassium oxalate was put in the bottom of the jar in order to prevent coagulation of the blood. During the bleeding the blood was stirred with an absolutely smooth glass rod. This was done very gently in order not to mix the blood with drops of paraffin. As a rule 3 to 4 cc. of blood were taken during a period of from 1 to 2 minutes.

2. Determination of the Oxygen Content of the Blood.—Van Slyke's method was employed. For each analysis 1 cc. of blood was used, and in all cases duplicate determinations were made. In one instance the Krogh modification of the Haldane-Barcroft method (differential method) was used because the amount of blood taken from the cutaneous incision was too small to allow duplicate determinations by Van Slyke's method. The total oxygen capacity was determined either by using a standardized Autenrieth colorimeter, or by saturating a sample of blood and determining the amount of oxygen. For this procedure only 1 cc. of blood was necessary because the blood was saturated within the Van Slyke apparatus as previously described (7). In Table I are given the figures for the hemoglobin value and for the total oxygen capacity.

EXPERIMENTS.

Experiment 1. Normal Resting Individual.—After the subject had rested on a bed for 5 minutes, a puncture of the left radial artery was made.[2] The moment that blood appeared in the syringe, blood was drawn from the right vena mediana cubiti. An incision was made in the tip of the second finger of the right hand without any previous freezing. 20 cc. of arterial blood were taken during a period of 5 seconds, 12 cc. of venous blood during 10 seconds, and 2 cc. of cutaneous blood during 45 seconds.[3] The results of the analyses are given in Table I and in Text-



TEXT-FIG. 1. Diagram showing the total oxygen-combining power of the blood (*T*), the oxygen content of the arterial blood (*A*), the oxygen content of the sample of blood obtained from an incision in the finger-tip (*C*), and the oxygen content of the blood from the vena mediana cubiti (*V*). All are given in volumes per cent (cubic centimeters of oxygen per 100 cc. of blood) and refer to Experiment 1 in Table I. The black part of the column indicates the amount of reduced hemoglobin, and the cross-hatching the amount of oxyhemoglobin. The total oxygen-combining power of the blood in this patient was 20, corresponding to a hemoglobin percentage of 108.

[2] The arterial puncture was done by Dr. George A. Harrop, Jr., of Baltimore.

[3] Of the five subjects, two (Nos. 1 and 2) are the authors. One (No. 4) was a private patient who volunteered. The other two were patients in the University Clinic (No. 3 was one of the nurses) who were willing to cooperate.

fig. 1. Both arterial and cutaneous blood were almost fully saturated with oxygen. The former showed 97.5 per cent and the latter 96.6 per cent of the total amount (20 volumes per cent) which the blood could absorb according to its hemoglobin content. The venous blood was 75 per cent saturated.

The percentage saturation of the arterial blood was in accordance with the results of previous investigators (Hürter (3), Barcroft (8), Stadie (4), Harrop (5), and Meakins and Davies (9)), who showed that in normal individuals arterial blood is from 90 to 100 per cent saturated. The value for venous saturation, incidentally, agreed with that previously found by one of us as the average saturation of venous blood in normal resting individuals (6). For cutaneous blood no previous determinations have been made.

Experiment 2. Normal Resting Individual.—After 15 minutes rest on a bed, an incision was made in the tip of the fourth finger of the left hand after previous freezing. During 2 to 3 minutes 4 to 5 cc. of blood were collected. During that period a sample of blood was drawn from the left vena mediana cubiti. Analysis of the two blood samples yielded results which were in accordance with those of the first resting subject. The cutaneous blood was almost completely saturated with oxygen (98 per cent), whereas the saturation of the venous blood was 65.8 per cent. The total oxygen capacity of the blood was found by direct determination to be 18.79 volumes per cent. No arterial puncture was made. We know that the saturation of the arterial blood in this case will be between 100 per cent, which is the total oxygen-combining power of the blood, and 98 per cent, which is the degree of saturation of the cutaneous blood. From the dissociation curve of the oxygen hemoglobin, and from the analyses of the arterial blood made by Hürter, Barcroft, Stadie, Harrop, and Meakins and Davies, we know that the saturation of the arterial blood is more likely to be 98 than 100 per cent. Therefore in this case too the cutaneous blood can be considered identical with the arterial blood as far as oxygen content is concerned.

Experiment 3. Normal Individual after Exercise.—After three unsuccessful attempts by Dr. Harrop to do arterial puncture, the subject before each attempt having run very quickly five times up and down a flight of stairs, the arterial puncture was given up. The subject again ran very quickly five times up and down a flight of stairs, and immediately after this an incision was made in the tip of the third finger of the right hand without previous freezing. 3 cc. of blood were taken during a period of 1 minute. Simultaneously a sample of venous blood was drawn from the arm of the same subject. The oxygen content of the cutaneous blood was found to be a trifle lower than that found in the previous experiments on resting individuals, but was within the normal limits of the oxygen content of arterial blood. The figure (94.3 per cent) approximates very closely that usually given as the average figure for normal resting individuals. The oxygen content of the

venous blood was the same as that usually found in normal resting individuals, but it differs from the values found in a series of experiments on the oxygen content of the venous blood after exercise in similar individuals (10). The analyses show that the largest possible discrepancy that can exist between the oxygen saturation of arterial and cutaneous blood is 5.7 per cent. The difference between the two figures is undoubtedly much smaller.

Experiment 4. Normal Individual after Exercise.—An incision was made in the tip of the fourth finger of the left hand after previous freezing. The finger was bandaged with gauze soaked in paraffin. After this the subject ran five times up and down a flight of stairs very quickly, taking 1 minute and 15 seconds. After the exercise his pulse was 132 and his respiration 40. 3 cc. of blood were taken during a period of 1 minute in the usual way from the incision already made. The cutaneous blood was in this instance also almost totally saturated (98.2 per cent). The saturation of the arterial blood must, of course, be between the total oxygen capacity, which was 22.18 volumes per cent, and the oxygen content of the cutaneous blood, which was 21.78 volumes per cent.

Experiment 5. Patient with a Large Exudate in the Left Pleura; an Almost Total Compression of the Left Lung.—A clinical and x-ray examination showed that the left lung was practically compressed. The patient was rather pale. No cyanosis was present. Pulse was 132 and respiration 58. Without previous freezing, two incisions, each 1 cm. long, were made in the tip of the fourth finger of the right hand, and simultaneously an incision was made in the right vena mediana cubiti. The saturation of the cutaneous blood was 93.2 per cent, which was somewhat lower than the average for normal resting individuals, but still within normal limits. The saturation of the venous blood was normal (72.7 per cent). The arterial blood must in this case have been saturated to at least 93.2 per cent. These figures and the result of the x-ray and clinical examinations make it certain that the left lung must have been compressed to such an extent that not only was the passage of air prevented, but also the blood flow. This furthermore is in accordance with the fact that the patient was not cyanotic (10).

Experiment 6. Patient with Compensated Mitral Stenosis, before and after Exercise.—After 30 minutes of absolute rest, an incision was made in the tip of the fourth finger of the left hand, the finger having previously been frozen. 4 cc. of blood were taken from the incision during a period of 2 minutes. At the same time a puncture was made in the left vena mediana cubiti. The pulse rate was 100 and the respiration 24. Then the finger was bandaged and the patient went slowly up stairs, one flight, and was put to bed. Immediately after this another sample of venous blood was taken from the same vein as before. An attempt was made to get a sample of cutaneous blood but without success on account of coagulation of the blood in the incision. Just after the patient had climbed the stairs the pulse rate was 132 and the respiration 36. During rest her lips were cyanotic, whereas the color of her finger-tips was more pinkish (erythrosed). After exercise the cyanosis was somewhat increased without becoming general. Her finger-tips were somewhat cyanotic. The analyses showed

that the cutaneous blood taken during rest was almost fully saturated with oxygen (98.2 per cent). We can therefore consider the cutaneous blood in this case to be identical with the arterial blood as far as the oxygen content is concerned. The experiment also shows that if any part of the lungs were not sufficiently ventilated (a few coarse râles were heard in the back of both sides) these parts could not have been passed by any blood, because had that occurred, the result would have been an abnormally high unsaturation of arterial and cutaneous blood. This, as the analyses show, was not found to be the case. Analyses of the two samples of venous blood taken before and after exercise yielded quite different results. At rest the venous blood was found to be 69.3 per cent saturated, which corresponds fairly well to the saturation of the average normal resting individual. This is also in accordance with the fact that no cyanosis of the fingers was present at that time. As already mentioned, however, the lips were cyanosed while the subject was resting. This cyanosis must necessarily be of peripheral (capillary, non-pulmonary) nature, and mainly due to deoxidation within the capillaries in the lips, because the arterial blood as shown was at least 98.2 per cent saturated. After the exercise, which was very slight, the venous blood contained only a small amount of oxygen, being 29.4 per cent saturated. This figure gives a venous unsaturation of 14.4 volumes per cent. At that time a slight cyanosis of the fingertips was observed. There seem to be two possible explanations of the heavy increase in the oxygen unsaturation of the venous blood after exercise: one, that it was due to insufficient oxidation of the blood in the lungs, the result of which would be increased unsaturation of the arterial and capillary blood; the other, that an increased deoxidation in the periphery had taken place. The latter explanation seems to be the more likely on account of the fact that no general cyanosis was observed after exercise. From previous investigations we know that cyanosis of pulmonary origin is usually more or less generalized; but in the case of this patient the cyanosis was not general. While she was climbing the stairs her hands and arms were kept perfectly quiet so that exercise could not have directly produced an increased deoxidation in the hands on account of increased local metabolism in the arm. If her cyanosis was of a peripheral nature and due to increased deoxidation in the capillaries of the hands, this deoxidation must have been due to a rather marked vascular constriction causing a decrease in the local blood flow. We have, in another series of experiments on normal individuals, observed a similar condition after heavy exercise (11, 12) (running quickly five times up and down one flight of stairs), and we have brought out different facts which seem to show that this increased oxygen unsaturation of the venous blood was due to slow local blood flow owing to vascular constriction in the arms which were at rest during the experiment. The vasoconstriction would tend to increase the blood flow in the active parts (lower extremities) during exercise. If the same explanation applies to this patient, it would show that a very slight amount of exercise brought her vasoconstrictory mechanism in the resting regions into activity. With a decrease in the blood flow in the resting parts the patient could perform the exercise with a smaller increase in the minute volume of the heart than would otherwise have been the case.

Experiment 7. Patient with Pulmonary Emphysema, Bronchial Asthma, and Chronic Bronchitis, before and after Exercise.—The patient had a chest of typical emphysematous shape. No absolute heart dullness was present, but apart from that the examination of the heart was normal. The lungs extended on the back to the twelfth rib, and on the right in front to the seventh rib. In both lungs a number of rhonchi and a few coarse râles were observed. No fine râles were present. The patient was not cyanotic. During rest the pulse was 96, and the respiration 24. After exercise the respiration went up to 32. The pulse rate was not observed. After 15 minutes rest on a bed an incision was made in the finger-tip after previous freezing. A sample of venous blood was taken at the same time. The patient then walked twice quickly up and down one flight of stairs. Immediately after exercise and without previous freezing, an incision was made in another finger of the same hand as before. Simultaneously a sample of venous blood was drawn from the same vein. The analyses of the blood taken during rest showed the same results that are found in normal resting individuals, the cutaneous blood being almost fully saturated with oxygen (97.9 per cent). Therefore, in this case it appears, first, that we are justified in considering the arterial and cutaneous blood as identical, at any rate as far as the oxygen content is concerned; and second, that the lungs, in spite of their pathological condition, allowed the blood to be normally saturated during its passage through the pulmonary circulation. This is in accordance with the results of M. Krogh's (13) determinations of the diffusion constant for the lungs in four resting emphysematous patients. In four instances this author's figures were within the limits which she previously had established as normal. After a rather slight amount of exercise, an oxygen unsaturation in the cutaneous blood of 2.56 volumes per cent was observed. This corresponded to a saturation of 89 per cent, a figure which is just below the lower limit of the oxygen saturation in the arterial blood in normal resting individuals. Determination of the saturation of arterial blood in normal individuals during exercise has been made by Harrop (5) and Barcroft and his collaborators (14). In both studies a slight decrease in the arterial oxygen saturation was found after very heavy exercise. If the cutaneous blood in our patient, also after exercise, can be considered identical with the arterial blood, our experiment might indicate that the patient's lungs were not quite capable of meeting the increased need for oxygen during the exercise. We do not think that the lower figure for the oxygen content of the venous blood after exercise can be attributed to the fact that no freezing was used in this instance (Table I).

Experiment 8. Normal Resting Individual after Inspiration of Air with a Low Oxygen Content.—The subject, the same as in Experiment 1, was made to rest quietly on a bed for 10 minutes, after which time he started to breathe from a spirometer which contained a gas mixture of the following composition: oxygen, 5.88 per cent; carbon dioxide 0.13 per cent; and nitrogen 93.99 per cent. The temperature was 16°C.; barometric pressure 776 mm.; oxygen tension 42.77 mm.; and carbon dioxide tension 0.935 mm. 75 seconds after the patient had begun to breathe this mixture the first signs of cyanosis of the lips, ears, cheeks, and fingers

were observed; and after 90 seconds the patient's skin showed general and very marked cyanosis. At this time a sample of venous blood from the right vena mediana cubiti was drawn, and three incisions were made, after previous freezing, in the fourth finger of the left hand. The drawing of the venous blood lasted 10 seconds, whereas it took 2 minutes to collect 5 cc. of cutaneous blood. During the final period of breathing from the spirometer the subject had a very unpleasant feeling of palpitation, air-hunger, and tingling in the extremities, and was very near unconsciousness. After the sample of cutaneous blood was drawn, the subject again started breathing atmospheric air. The respirations were very frequent and very deep, and he felt an immediate relief. The cyanotic skin color disappeared very quickly and was not observed 20 to 25 seconds after beginning to breathe normal air. At that time another sample of cutaneous blood was taken from the incision previously made. It took 30 seconds to collect this blood. The first sample of cutaneous blood showed an unsaturation of 6.9 volumes per cent, which corresponds to 63 per cent saturation. This value is, of course, below the limit for the oxygen content of the arterial blood. On account of the low oxygen tension in the lungs the upper limit for the arterial saturation is much below the total oxygen-combining power. By means of the dissociation curve of the oxyhemoglobin, and the figures for the oxygen tension in the spirometer, we calculate that about 70 per cent would approximate the upper limit for saturation of the arterial blood. Analysis of the venous blood drawn during respiration from the spirometer showed almost the same amount of oxygen that was found in the cutaneous blood. This is probably due to the fact that the venous blood was drawn during a very short period in the first part of the experiment, whereas the cutaneous blood also contains blood from the last part of the experiment; that is, during the time that the arterial unsaturation had reached its highest value. On the other hand, it is not unlikely that the deoxidation in the capillaries was relatively small on account of increased blood flow. Half of the sum of the oxygen unsaturation of the cutaneous (arterial) and the venous blood is 8 to 9 volumes per cent, which is well above the threshold value at which cyanosis usually appears (10). That the cyanosis was found to be generalized is in accordance with its arterial (pulmonary) pathogenesis. The fact that cyanosis of pulmonary origin is more intense in some regions than in others is due to local conditions; for instance, a greater degree of deoxidation in some regions than in others, a richer network of superficial capillaries, a larger filling of the capillaries with blood, or more opened up capillaries (A. Krogh).

In the second cutaneous blood sample normal saturation was found. The change from a marked cutaneous unsaturation to normal condition took place in less than ½ minute after the subject started to breathe normal air. This shows that the circulation of the blood must have been very rapid and that the alveoli of the lungs immediately were supplied with oxygen-rich air. The experiment is in accordance with a similar one done by one of us in 1919 on the same subject (10). No cutaneous blood was drawn at that time.

DISCUSSION.

From the experiments it is evident that the oxygen content of the cutaneous blood and of the arterial blood may show almost identical values. Not only was this true of normal individuals before and after exercise, but it was also found to hold under different pathological conditions. This is, as far as we can see, sufficient to show that, at any rate in some cases, we might obtain similar information from samples of cutaneous and of arterial blood. In the description of the experiments several examples were given of the kind of problem in which examination of cutaneous blood would be of value. We think that in cases in which it is necessary to repeat the blood examination very often, or when for some reason or other it is impossible to obtain arterial blood, it will be especially useful to know that the same information may be obtained by analyzing cutaneous blood. On the other hand, it is admitted that it is not an easy thing to obtain 2 to 3 cc. of blood from an incision in the finger-tip without using stasis. Rather large, and, if freezing is not applied, somewhat painful incisions will be necessary. Two improvements would, we think, be necessary to make the method of general use: one, to perform the analyses for oxygen content with smaller amounts of blood, for instance 0.2 cc. instead of 1 cc. for each analysis as in Van Slyke's apparatus (in this case less than 0.5 cc. would be necessary for other duplicate determinations; such small amounts are, of course, sufficient if the differential method is used which we have employed in only one instance—No. 1); the other improvement would consist in finding a substitute for the method of drawing the blood under oil, for this procedure makes it necessary to draw a larger amount of blood than is needed for the analyses. As to the first point, it will be necessary to find out how much we can decrease the amount of blood taken from the incision and still obtain the same results. As to the second point, we have so far been unsuccessful in developing a method of drawing a small amount of blood from a cutaneous incision directly into a small tube without having the blood come into contact with the air.

Attention should be called to a problem which is not only of theoretical but also of practical importance. It is whether the blood which can be obtained from the cutaneous incision, and which we in

our cases found to be identical with the arterial blood, is genuine arterial blood, that is practically all coming from the arteries themselves, or whether it is to be looked upon as only functional arterial blood, that is arterial blood mixed with a considerable amount of non-deoxidized blood from the capillaries and veins. Both explanations might be correct. An incision in the tip of the finger necessarily severs a number of vessels including small arteries, capillaries, and small veins. It is, however, *a priori* not impossible that after the incision is made, the same conditions would be present that we would find if a slanting water pipe was severed through which water flows from a reservoir with high pressure to a reservoir with low pressure. After cutting such a pipe the water would, of course, only flow out from that part of the pipe that is connected with the reservoir with high pressure. The water in the other part of the pipe would continuously flow away from the place where the pipe was cut. It is known that the statical and other conditions in the circulatory system are different from the conditions in a water pipe, but still it is not an absurdity to suppose that the negative, or at any rate always very low pressure in the central thoracic veins can prevent anything but a negligible amount of blood coming out from the venous side of the severed vessels. The tendency of the veins to collapse would probably act in the same direction. In any case, if no stasis is applied, the difference in pressure in the direction of the blood flow in the arterial and venous system must necessarily bring it about that by far the largest amount of blood taken from the cutaneous incision comes from the arteries. On the other hand, immediately after the incision is made, some blood must necessarily come from the severed veins and capillaries. This blood is probably very quickly washed out from the incision and is contained in the first drop of blood coming out. We have in some, but not in all instances noticed that the first drop of blood coming from the incision is dark, and the following drops very light, indicating that the first drop contains a not negligible amount of venous blood. Even if one makes it a rule, as we have done, to discard in each experiment the first drop of blood, it is possible that a very small amount of blood drawn might contain enough venous blood to make an appreciable difference between the cutaneous and arterial blood. The smallest amount we have taken

has, as mentioned, been 2 cc. The second explanation was that blood obtained from a contaneous incision is derived not only from the arteries, but also, to a considerable extent, from capillaries and veins. It is therefore mixed blood. There are two explanations of the fact that it is arterial in respect to its oxygen content. In the first place, this can be due to a vasodilatation of the region where the incision is made. It is a well recognized fact (1) that mechanical, chemical, or thermal irritants applied to the skin result in a dilatation of the vessels. An increase in the local blood flow and a decrease in local deoxidation must necessarily follow such a vascular dilatation. If an incision is made in such a hyperemic region, quite apart from whether the blood comes from the arteries, capillaries, or veins, it is quite likely that the blood will not differ appreciably from the arterial blood. In the experiments, the finger-tips were in all instances cleaned with alcohol, from 5 to 15 minutes before the incision was made. Furthermore, in some instances (Table I) the skin was frozen before incision was made. The figures in Table I seem to indicate that previous freezing did not have any influence on the results. Whether or not the rubbing with alcohol could be made responsible for the high oxygen content of the cutaneous blood, we are unable to say; but it does not seem very likely, because the alcohol, in some instances, was applied several minutes before the incision. It seems to be more likely that if a vasodilatation takes place, it might be due to the incision itself. An arterialized state of the cutaneous blood could, however, be caused in quite another way. The ratio between the capillary blood flow and the metabolism of the skin might *normally* be very high, so high that no appreciable deoxidation per blood unit takes place. If this were the case, we could explain not only the fact that the oxygen content of the cutaneous blood equals that of the arterial blood, but also an (unpublished) observation we have made in several instances that cyanosis often seems to follow the arterial unsaturation more closely than it does the venous. Such a condition would not be difficult to bring into harmony with one of the chief functions of the skin—the regulation of the temperature. The main difference between the arterial and venous blood of the skin should then be the temperature and not the content of substances relating to the metabolism. On the other hand,

the fact that the first drop of cutaneous blood is often darker than the rest argues against this explanation. The peripherally caused form of cyanosis, that is the cyanosis due to increased deoxidation of capillary blood, also points against such an assumption of the skin circulation.

At first glance it would also seem quite contradictory to this last explanation that the venous arm blood was normally deoxidized in our experiments. No definite conclusions can be drawn from this, however, because the cubital vein drains from other regions than this from which the blood is drawn. Whether one or the other of these explanations contains the truth, it is, at this time, impossible to say.

The identity between the arterial and cutaneous blood has, in the cases examined, only been proved to be true for the oxygen content. There can, however, be little doubt that identity can be extended to include other substances in the blood which pass the capillary wall, for instance salt, sugar, uric acid, etc., because we think that oxygen is the finest indicator of the metabolic interchange between the tissues and the capillary blood. Probably this identity also includes the reaction of the blood which, as is well known, differs in the arteries and veins.

SUMMARY.

1. A procedure is devised whereby cutaneous blood (so called capillary blood) from a finger-tip can be obtained for gas analyses without coming in contact with the air.

2. Determination was made of the oxygen content of the arterial, cutaneous, and venous blood respectively from a normal resting individual, the arterial and cutaneous blood showing the same oxygen content (97.5 and 96.6 per cent of the total oxygen-combining power of the blood). Venous blood drawn simultaneously was 75 per cent saturated.

3. Using the fact that there is a maximum value for the oxygen-combining power of the blood, we have shown, without doing arterial puncture, that under different conditions (normal and pathological individuals, resting and after exercise) the cutaneous blood and the arterial blood are almost identical as far as the oxygen content is concerned.

4. We think that we are justified in extending the identity, found between the oxygen content of the arterial and cutaneous blood, to

other substances in the blood, for instance sugar, salt, uric acid, etc., and also to the reaction of the blood.

5. We are unable to say whether this identity between cutaneous and arterial blood is always true; for example, in a patient with increased venous pressure.

6. In all the experiments we have discarded the first drop of blood, which in some instances was darker than the rest, and always used at least 2 cc. of cutaneous blood. Whether the same results would be obtained with a very small amount of blood, for instance 0.2 to 0.4 cc., we do not know.

7. The experiments show that unless the perfusion of the skin has been extremely great during the experiment, samples of blood obtained from an incision in the skin (of the finger) cannot represent the the true capillary blood. The neutral expression cutaneous blood seems therefore for the present preferable to the term capillary blood for samples of blood obtained by cutaneous incisions.

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STUDIES ON THE BIOLOGY OF STREPTOCOCCUS.

II. Antigenic Relationships between Strains of Streptococcus hæmolyticus Isolated from Scarlet Fever.

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In a previous publication, the author (1) has set forth the results of a study of the agglutination reactions of twenty-five strains of *Streptococcus hæmolyticus* isolated from the throats of patients suffering from scarlet fever. Of these strains, twenty were agglutinated at equal titers by four separate immune sera prepared by the immunization of rabbits to individual strains of scarlatinal streptococci. With the exception of four strains, none was agglutinated by any of four antistreptococcic sera obtained by immunization of animals to strains of *Streptococcus hæmolyticus* isolated from diseases other than scarlet fever. These facts suggest that certain hemolytic streptococci found in the throats of scarlet fever patients constitute a single biological group.

The simultaneous studies of Tunnicliff (2) reveal a similar relationship between strains of *Streptococcus hæmolyticus* associated with scarlet fever. More recently, Gordon (3) has found that eighteen strains of hemolytic streptococcus isolated from scarlatina are identical in their agglutination reactions. None of these strains absorbed the agglutinins from immune sera prepared from certain other types of hemolytic streptococcus, designated by him as Types I and II. On the basis of this evidence, Gordon concludes that the streptococci from the throat secretions in scarlet fever constitute a group serologically distinct from other varieties of *Streptococcus pyogenes*.

The occurrence of hemolytic streptococcus in the throats of patients with scarlet fever is well known and it seems unnecessary to review the literature on the subject at the present time. The most recent observations indicate that *Streptococcus hæmolyticus* is almost constantly

associated with this disease. In the author's series of cases hemolytic streptococcus has been obtained from the throats of 100 per cent of all patients cultured during the 1st week of illness. In those individuals from whom cultures were taken during the first few days of the disease the above organism was found to predominate on the plates. After the 1st week of the disease the number of hemolytic streptococci diminishes rapidly, although great numbers may still be present as late as the 12th day. The average length of time that these organisms are carried in the throat secretions is 10 days. Several examples of the carrier condition have been encountered, and in one individual the specific streptococcus was found to be present in considerable numbers in the throat 1 year after the attack of scarlet fever.

No morphological or cultural characteristics that seem to differentiate the hemolytic streptococcus associated with scarlatina from other types of *Streptococcus pyogenes* have been demonstrated. Many observers have emphasized the variability of the scarlet fever streptococci, and because of this have regarded them as secondary invaders. Such variations as occur seem in all likelihood to be related to inconstancy of the cultural environment rather than to fundamental biological differences. The more recent studies of this organism indicate that differences of this character are of very little value in dividing the group into its specific varieties.

The organisms which have been examined in the present series have been characterized on isolation by wide variations in growth characters.

Some have been very minute and others relatively large. Some grow in short chains, others in long ones. A curious tendency to loss of Gram positiveness has been observed. Although the organisms originally obtained from the peritoneal exudates of mice or from blood agar plates retain the Gram stain, the same strains in transplants from old stock cultures or after cultivation in broth may be continuously Gram-negative. Certain strains manifest a tendency to develop so called involution forms on cultivation in broth media. Many of the individual cocci become from two to three times their original size and appear swollen. Sometimes a culture will become almost entirely converted into forms of this character. Cultivation of such strains in enriched media or even animal passage fails to restore the normal morphology. Coincident with this change in size the strain may become inagglutinable in an immune serum which previously agglutinated it in high titer. One strain (SH26) exhibited this irregular morphology

from the time of isolation and was continuously inagglutinable. The appearance of the colony on blood agar plates is fairly constant, a small moist slightly raised colony with a smooth margin, appearing through a hand lens as an evenly, finely granular dewdrop. About the colony there is a zone of complete hemolysis, of about the same width as the diameter of the colony. The extent of hemolysis, however, varies with different conditions of the media. In some instances the colonies are extremely minute with wide coalescing areas of hemolysis, and in others the zone of hemolysis is quite narrow—from one-half to one-third of the diameter of the colony. Subsequent cultivations indicate that these differences are not constant and that they are dependent upon slight unexplained variations in media or in growth activity.

The hemolytic streptococci from scarlet fever show the same tendency to granular growth in broth that is such a constant feature of other types of *Streptococcus hæmolyticus*. Great variations, however, in this characteristic are observed.

All strains when first isolated tend to flocculate when cultivated in broth or when exposed for a few hours in the water bath to a temperature of 55°C. Most strains will grow diffusely in broth after repeated transfers in this medium. Some require relatively few transfers and others from twenty to thirty passages before stable suspensions can be obtained. Certain strains remain persistently granular even after daily passage in broth media for long periods of time. Occasionally a strain will grow diffusely for a while and then again become granular although there has been no recognizable change in the character of the culture medium or in the method of handling the organism. Once an organism acquires the character of diffuse growth sufficiently stable suspensions can usually be prepared to carry out the agglutination reaction at 55°C.

Certain qualities of the medium promote diffuseness of growth.

The addition of glucose or of ascitic fluid causes the cultures to grow luxuriantly and diffusely at incubator temperature, but when such organisms are exposed to normal serum, in the water bath at 55°C., they tend to flocculate, obscuring the results of the agglutination tests. It has been found that flocculation occurs when the sugar concentration is above 0.2 per cent, and that when the concentration is below 0.1 per cent the organisms fail to grow sufficiently to give a satisfactory suspension. Slight variations in the sodium chloride or protein content of broth have no demonstrable effect on the luxuriance or diffuseness of growth. In broth made of meat extract the organisms grow poorly or flocculate, and any appreciable variation from a final hydrogen ion concentration of the medium after sterilization of pH 7.5 results in insufficient growth or flocculation, or usually both. Certain difficulties have been traced to the use of tap water instead of distilled water in the preparation of the medium. It has been found that the addition of 1 or 2 drops of boiled tomato juice to a tube of broth (as used by Thjötta

and Avery (4)) facilitates the growth of the organisms, and does not interfere with agglutination. Passage of autoagglutinable strains through mice sometimes renders them diffuse, but because of the observations of Aronson (5), Meyer (6), and others, that passage through animals makes cultures less agglutinable, the strains used in the present experiments were not passed through animals but grown continuously in broth.

A great variety of fermentation reactions in carbohydrate broth of hemolytic streptococci isolated from the throats of scarlet fever patients has been recorded by different workers. This has led many to believe that they were dealing not with a single type but with a great number of varieties of *Streptococcus hæmolyticus*.

It is, of course, a question how much significance should be attached to these differences in reaction to sugars, particularly in view of the lack of uniformity of the methods employed. Using for convenience the nomenclature of Holman (7) in his classification of streptococci on a basis of carbohydrate fermentation reactions, and considering only strains of hemolytic streptococci isolated from the throats of scarlet fever patients, we find, for example, that Ruediger (8) in a study of thirty-seven such strains, groups some as *Streptococcus infrequens* (lactose, salicin, mannitol fermenters), and others as *Streptococcus pyogenes* (lactose, salicin, non-mannitol fermenters); Kligler (9) encountered *Streptococcus infrequens* and Andrewes and Horder (10) found ten strains of *Streptococcus anginosus* (lactose, non-salicin, non-mannitol fermenters) and three of *Streptococcus subacidus* (non-lactose, non-salicin, non-mannitol fermenters). Floyd and Wolbach (11) report eleven strains of *Streptococcus anginosus* and five of *Streptococcus subacidus*. Similar results have been reported by other investigators. Tunnicliff's (2) strains were with few exceptions *Streptococcus pyogenes*. These rough classifications do not take into account many differences in reaction to saccharose, raffinose, and other less commonly used sugars.

EXPERIMENTAL.

Carbohydrate Fermentation.

The carbohydrate fermentation reactions of the present series of throat strains are presented in Table I.

It is seen that of 64 throat strains examined, 53 (82.8 per cent) are true *Streptococcus pyogenes* (Holman), and if one accepts the opinion of Gordon and others that certain strains of *pyogenes* occasionally ferment mannitol, one may be justified in assuming that six additional strains, or 59 in all (92 per cent), are *Streptococcus pyogenes*. Of the

TABLE I.

Carbohydrate Fermentation by Strains of Hemolytic Streptococci Isolated from the Throats of Scarlet Fever Patients.

Strain No.	Lactose.	Salicin.	Mannitol.	Inulin.	Raffinose.	Strain No.	Lactose.	Salicin.	Mannitol.	Inulin.	Raffinose.
11	+	+	−	−	−	74	+	+	−	−	−
12	+	+	−	−	−	75	+	+	−	−	−
13	+	+	−	−	−	76	+(?)	+	−	−	−
14	+	+	−	−	−	77	+	+	−	−	−
15	+	+	+	−	−	78	+	+	−	−	−
16	+	+	−	−	−	79	+	+	−	−	−
20	+	+	−	−	−	80	+	+	−	−	−
23	+	+	−	−	−	81	+	+	−	−	−
24	+	+	−	−	−	82	+	+	−	−	−
25	+	+	+	−	−	83	+	+	−	−	−
26	+	+	−	−	−	84	+	+	−	−	−
27	+	+	−	−	−	88	+	+	−	−	−
28	+	+	+	−	−	89	+	+	−	−	+
29	+	+	+	−	−	90	+	+	−	−	−
30	+	+	−	−	−	91	+	+	−	−	−
35	+	+	+	−	−	93	+	+	−	−	−
36	−	+	−	−	−	94	+	+	−	−	−
38	+	+	−	−	−	95	+	+	−	−	−
39	+	+	−	−	−	98	+	+	+	−	−
45	+	+	−	−	+	99	+	+	−	−	−
47	+	+	−	−	−	201	+	+	−	−	−
50	+	+	−	−	−	203	+	+	+	−	+
53	+	+	−	−	−	205	+	+	−	−	−
54	+	+	−	−	−	207	+	+	−	−	−
56	+	+	−	−	−	208	+	+	−	−	−
62	+	+	−	−	+	209	+	+	−	−	−
65	+	+	−	−	−	210	+	+	−	−	−
66	+	+	−	−	−	211	+	+	−	−	−
67	+	+	−	−	−	212	+	+	−	−	−
68	+	+	−	−	−	213	+	+	−	−	−
69	+	+	−	−	−						
70	+	+	−	−	−						
72	+	+	−	−	−						
73	+	+	−	−	−						

0.1 per cent carbohydrate meat infusion broth was used containing Andrade indicator. Incubated 1 week; readings made daily.

+ indicates fermentation, − no fermentation.

remaining five strains, four ferment raffinose, which puts them in the group of *Streptococcus salivarius*, presumably a saprophytic type. It is interesting to note in this connection that, as shown below, three of these raffinose-fermenting strains differ in their reactions to agglutinating sera from the other strains. In brief, it may be concluded from these results that most of the strains belong to the type of *Streptococcus pyogenes*, and do not differ in their carbohydrate fermentation reactions from strains isolated from acute tonsillitis, pharyngitis, erysipelas, and certain septic processes. On the other hand, our experience does not demonstrate the existence of a great variety of carbohydrate-fermenting types. Some strains are slow fermenters, and it may be that some of the variations previously reported are due to incomplete observations. Studies with the less common sugars have not been made.

Agglutination Tests.

Numerous investigators have studied the agglutination reactions of streptococci. The results on the whole have been rather conflicting due largely no doubt to differences of method and inherent technical difficulties.

Moser (12) and Moser and von Pirquet (13) have prepared polyvalent sera from horses, using the streptococcus of scarlet fever as antigen. They studied the capacity of such sera to agglutinate specifically various strains of scarlatinal streptococci. The latter strains were agglutinated in dilutions of 1:1,000 or over, whereas hemolytic streptococci from other sources were not specifically agglutinated. As a consequence of these observations the authors believe that the streptococcus of scarlet fever differs from similar strains isolated from instances of erysipelas, phlegmon, puerperal sepsis, etc. Meyer (6) and later Rossiwall and Schick (14) have in general confirmed the results of Moser and von Pirquet.

On the other hand, Hasenknopf and Salge (15), Aronson (5), and also Neufeld (16) have been unable to duplicate the above results. They failed to find definitely specific relationships between the streptococci of scarlet fever and are of the opinion that it is not possible to differentiate between types of hemolytic streptococci by means of the agglutination reaction.

The discordant results briefly reported are no doubt due in part to technical difficulties encountered in the effort to study specific agglutinations of streptococcus and in part to the variety of methods

employed. The tendency of these organisms to agglutinate spontaneously in normal sera, and in broth and saline suspensions is very confusing. In addition, animal passage and long continued growth in artificial media may induce variations in agglutinability. Moreover, the methods of different investigators have not been strictly comparable. For these reasons it has been impossible up to the present time to answer conclusively the question as to whether or not the streptococci of scarlet fever form a specific immunologic type.

In a recent publication, Dochez, Avery, and Lancefield (17) have described a technique for the agglutination of streptococcus which has made it possible to differentiate between types of hemolytic streptococci isolated from a variety of respiratory diseases. Their procedure has been used in the present study. Its essential points are the use for purposes of culture of a meat infusion broth the reaction of which remains at or near pH 7.5. No sodium chloride is added and in such medium most strains of streptococcus can be grown diffusely. Broth of the same character and reaction is used in all the necessary dilutions both of organisms and of sera. The test suspensions are incubated for 1½ hours in a water bath at 55°C. and then read.

The sera were prepared by inoculating rabbits intravenously at 2 or 3 day intervals with increasing doses of heat-killed broth cultures, starting with 0.2 cc. and increasing to 2 cc. All rabbit sera were tested before the first inoculation in order to guard against spontaneous agglutination by normal rabbit sera. The above method produced sera with a titer of over 1:2,560. It was noted that the serum immediately after separation from the clot showed an agglutinating titer markedly lower than after standing for 48 hours or longer in the ice chest, so only sera have been used which have remained at least 48 hours on ice. Such sera, however, lose their agglutinins after standing for a few months.

The results of the first series of agglutination tests have been reported (1). They are recapitulated in Table II, with the addition of a few strains studied shortly thereafter.

Table II shows that none of five non-scarlatinal antistreptococcic sera (R.Sm.3, R.Sm.23, R.Sm.32, R.Sm.60, and R.Sm.84, the type sera of Dochez, Avery, and Lancefield) agglutinates any of the thirty-three strains of streptococcus isolated by the author from the throats

TABLE II.

Agglutination of Hæmolytic Streptococci from the Throats of Scarlet Fever Patients with Non-Scarlatinal and Scarlatinal Antistreptococcic Sera. Series 1.

Scarlatinal streptococcus strains.	Antistreptococcic sera.								
	Non-scarlatinal sera.					Scarlatinal sera.			
	R.Sm.3.	R.Sm.23.	R.Sm.32.	R.Sm.60.	R.Sm.84.	R.Sm.273.	Sc.Sm.23.	Sc.Sm.24.	Sc.Sm.25.
11	–	–	–	–	–	+++++	++++	+++++	+++++
12	–	–	–	–	–	++++	+++++	++++++	+++++
13	–	–	–	–	++++	++++	++++	+++++	+++
14	–	–	–	–	++++	+++	+++++	++++++	++++
15	–	–	–	–	–	+++++	++++	+++++	+++++
16	–	–	–	–	++++++	+++++	++++++	++++++	++++++
20	–	–	–	–	–	–	–	–	–
23	–	–	–	–	–	++++	++++++	+++	++++++
24	–	–	–	–	–	++++	+++++	++++++	++++++
25	–	–	–	–	+++++	++++++	++++++	+++++	+++++
26	–	–	–	–	–	–	–	–	–
27	–	–	–	–	–	++++	+++	+++++	++++
28	–	–	–	–	–	++++	++++	++++++	++++++
29	–	–	–	–	–	++++	+++	++++++	++++++
35	–	–	–	–	–	+++	+++	+++	++
36	–	–	–	–	–	–	–	–	–

38	–	–	–	–	–	+++++	++++	+++++	++++++
39	–	–	–	–	–	+++++	++++	+++++	++++++
45	–	–	–	–	–	–	–	–	–
47	–	–	–	–	++++	+++++	+++	++++++	+++++
50	–	–	–	–	+++++	++++++	++++	++++	++++++
53	–	–	–	–	–	++++++	++++	++++++	+++++
56	–	–	–	–	–	++++	+++	++++++	+++
62	–	–	+++	–	–	–	–	–	–
65	–	–	–	–	–	++++++	++++	++++++	++++++
66	–	–	–	–	–	0	+++++	0	++++++
67	–	–	–	–	–	++++	++++++	+++	++++++
68	–	–	–	–	–	++++	+++	++++	+++++
69	–	–	–	–	+++	+++	+++	++++	++++
70	–	–	–	–	–	0	++++	0	+++++
72	–	–	–	–	+++	++++++	0	+++++	0
73	–	–	–	–	–	–	–	–	–
R.273	–	–	–	–	–	++++++	+++++	+++	+++++

Nos. R.Sm.3, R.Sm.23, etc., indicate the Rockefeller type strain antistreptococcic sera used, obtained through the courtesy of Dr. O. T. Avery from The Rockefeller Institute for Medical Research. These were the same type sera which Dochez, Avery, and Lancefield used in their streptococcus classification experiments. The Rockefeller Type Serum 273 was made from a strain isolated from a suppurative gland in a case of scarlet fever. The other type strains were from respiratory infections.

Nos. Sc.Sm. 23, 24, and 25 were sera made from the author's Strains 23, 24, and 25, isolated from the throats of cases of scarlet fever.

In Tables II to IV, XIII, and XV + indicates agglutination present in a dilution of 1:20, ++ in dilution of 1:40, +++ in 1:80, ++++ in 1:160, +++++ in 1:320, ++++++ in 1:640. Higher dilutions were deemed unnecessary. 0 indicates test not made, – no agglutination.

of scarlet fever patients, with the exception of eight strains agglutinated by Serum R.Sm.84, and Strain 63, agglutinated by Serum R.Sm.32. Each of the scarlatinal streptococcic sera used agglutinates twenty-eight out of thirty-four strains isolated from patients with scarlet fever. It is of some interest that of the six scarlet fever strains not agglutinated by scarlatinal antisera, Strains 36, 45, and 62 differ from the type in their fermentation reactions. Strain 26 persistently showed large numbers of involution forms, a phenomenon characteristic of inagglutinable strains. Strain 54, erroneously included in the series first reported, has been omitted from Table II. In a few instances individual agglutination tests have not been made because of deterioration of the sera during the summer months.

In order to confirm the results reported in Table II an additional series of strains of streptococcus has been isolated from fresh cases of scarlet fever. New sera have been prepared by the immunization of rabbits and the specific agglutinating power of these sera has been tested. The results are summarized in Table III.

Of the twenty-four new strains studied, twenty are agglutinated specifically by antiscarlatinal sera. One strain (No. 79) displays some irregularity of agglutination, and three (Nos. 84, 203, and 205) fail completely to agglutinate. If the statistics reported in Tables II and III are combined it is found that 80 per cent or more of strains of streptococcus from scarlet fever are agglutinated by the specific sera employed. In all, the various strains were tested with ten different monovalent antistreptococcic sera. On the other hand, these same strains are not agglutinated by antistreptococcic sera prepared from hemolytic streptococci isolated from diseases or conditions other than scarlet fever.

A study of the capacity of immune sera prepared from the scarlatinal streptococcus to agglutinate streptococci from a variety of sources has also been made. The results of this study are shown in Table IV.

This table demonstrates that streptococci isolated from individuals not having scarlet fever or removed from contact with this disease do not agglutinate in antiscarlatinal sera. Of the five strains showing agglutination (Nos. 112, D10, 10, 55, and 57), one (No. 112) was from a chronic carrier, two (Nos. 10 and D10) were from recent contacts,

one (No. 55) from an infected mastoid shortly before the development by the patient of a typical scarlatinal rash followed by desquamation, and one (No. 57) from the throat of an individual suspected of having scarlet fever. In general then it would seem that hemolytic streptococci isolated from scarlet fever constitute a specific type which can be separated by the reaction of agglutination from hemolytic streptococci from other sources.

A number of studies have previously been made of the power of the serum of convalescents from scarlet fever to agglutinate the homologous streptococcus. The results have been irregular and conflicting and are of no great significance. In spite of these apparent failures to demonstrate a positive agglutination reaction the author has studied a few such sera. The results are presented in Table V. The degree of agglutination obtained in a few instances is considerable but the range so limited that positive deductions as to specificity are unwarranted.

Absorption of Agglutinin Tests.

Specific absorption of agglutinins by streptococcus is a procedure attended by certain technical difficulties. The high temperature at which the reaction must be carried out causes a considerable loss of agglutinating power by the immune sera employed. The use, however, of a very heavy suspension of bacteria for purposes of absorption so shortens the period of incubation that no great deterioration occurs. The sediment of 50 cc. of a 24 hour broth culture of streptococcus was added to each cubic centimeter of a dilution of 1:5 of the serum to be absorbed. The mixtures were incubated at 55°C. for 2 hours with frequent shaking and then the organisms were removed by centrifugalization. The normal sera used as controls were treated for a similar period of time. The results are shown in Table VI.

From this table it is seen that a scarlet fever streptococcus removes from an homologous serum the agglutinins for the particular strain used in its production and for other scarlet fever strains as well. On the other hand, a non-scarlatinal streptococcus does not absorb these agglutinins. Furthermore, a scarlatinal streptococcus fails to absorb the agglutinins from a serum prepared by immunization to a

TABLE III.

Agglutination of Hemolytic Streptococci from the Throats of Scarlet Fever Patients with Monovalent Scarlatinal and Non-Scarlatinal Antistreptococcic Sera. Series 2.

Scarlatinal streptococcus strains.	Antistreptococcic sera.								
	Scarlatinal sera.							Non-scarlatinal sera.	
	Sm.15a.	Sm.15b.	Sm.25.	Sm.53a.	Sm.53b.	Sm.76a.	Sm.76b.	Sm.102.	R.Sm.60.
74	++++++	++++++	++++++	++++++	++++++	++++++	0	–	0
75	++++++	++++++	++++++	+++++	++++	++++++	++++	–	0
76	+++++	+++++	++++++	0	+++	++++++	++++++	–	0
77	++++++	+++++	++++++	++++++	+++	++++++	0	–	0
78	++++++	++++++	++++++	–	++++++	++++++	++++++	–	0
79	–	0	++++	0	0	–	0	–	0
80	++++++	++++++	++++++	++++++	++++++	++++	++++	–	0
81	++++++	++++++	++++++	++++++	++++++	++++++	+++++	–	0
82	++++++	++++++	++++++	++++++	+++++	++++++	+++++	–	0
83	–	+++++	++++++	++++++	0	++++++	++++	–	0
84	–	–	–	–	–	–	0	–	0
88	++++++	++++++	++++++	++++++	++++++	++++	++++	–	0
89	++++++	++++++	++++++	++++++	++++++	++++	++++	–	0
90	++++	++++	++++	++++++	++++++	++++++	0	–	0
91	++++++	++++++	++++	++++++	+++++	0	0	–	0
93	++++++	++++++	0	+++++	0	+++	0	–	0

94	+++++	++++	++++++	+++	++++++	++++	0	–	0
95	++++++	++++	++++	+++	+++	++++++	0	–	0
98	++++++	++++	0	++++	0	0	0	–	0
99	++++++	0	0	0	0	++++++	0	–	0
201	++++++	0	0	0	0	++++++	0	–	–
203	–	0	0	0	0	–	0	–	–
205	–	0	0	0	0	–	0	–	–
207	++++++	0	0	0	0	++++++	0	–	–

Serum Sm.102 was a monovalent serum from a rabbit immunized with hemolytic streptococcus, Strain 102, isolated from the throat of a case of acute follicular tonsillitis. Serum R.Sm.60 was the Rockefeller type 60 serum.

TABLE IV.

Agglutination of Hemolytic Streptococci from Non-Scarlatinal Sources by Monovalent Scarlatinal Antistreptococcic Sera.

Non-scarlatinal strepto-coccus strains.	Source.	Scarlatinal antistreptococcic sera.			
		Sm.15a.	Sm.25.	Sm.53a.	Sm.76.
63	Blood culture, erysipelas.	–	–	–	0
101	Pus, osteomyelitis.	++++	–	–	0
103	" maxillary sinusitis.	–	–	–	0
104	" abscess of finger.	–	–	–	0
105	Cervical culture, puerperal sepsis.	–	–	–	0
106	Pus, abscess.	0	–	–	0
115	" empyema.	–	–	0	–
102	Tonsillitis.	–	–	–	0
111	"	–	–	0	–
112	"	++++++	+++	++++	0
113	"	–	–	–	0
114	"	–	–	–	0
D1	Normal throat, scarlet fever contact.	–	–	–	–
D3	" " " " "	–	–	–	–
D4	" " " " "	–	–	–	–
D5	" " " " "	–	–	–	–
D6	" " " " "	–	–	–	–
D7	" " " " "	–	–	–	–
D8	" " " " "	–	–	–	–
D9	" " " " "	–	–	–	–
D10	" " " " "	++++++	++	+	+++++

Non-scarlatinal strains previously recorded (1).	Source.	R.Sm.273.	Sm.25.		
4	Pus, cervical abscess.	−	−		
5	" " "	−	−		
32	" mastoid.	−	−		
37	Blood culture, septicemia.	−	−		
40	" " "	−	−		
49	" " erysipelas.	−	−		
55	Pus, mastoid, prescarlet fever.	++++++	+++++		
58	" "	−	−		
59	" abscess of jaw.	−	−		
6	Tonsillitis.	−	−		
9	"	−	−		
10	" (scarlet fever contact).	++++	+++++		
21	"	−	−		
22	"	−	−		
31	"	−	−		
41	"	−	−		
57	" (scarlet fever suspect).	++++++	++++++		

TABLE V.

Agglutination of Hemolytic Streptococcus from the Throats of Scarlet Fever Patients with Scarlatinal Convalescent Sera.

Scarlatinal streptococcus strains.	Convalescent scarlatinal sera.	Dilution.					
		1:8	1:16	1:32	1:64	1:128	1:256
12	Sm.62 (6th day).	−	−	−	−	−	−
24	" 62 (6th ").	−	−	−	−	−	−
25	" 62 (6th ").	−	−	−	−	−	−
28	" 62 (6th ").	−	−	−	−	−	−
11	Sm.62 (18th day).	++	+	−	−	−	−
12	" 62 (18th ").	++	+	+	−	−	−
14	" 62 (18th ").	+	+	−	−	−	−
15	" 62 (18th ").	++	++	+	+	−	−
23	" 62 (18th ").	++	+	+	−	−	−
24	" 62 (18th ").	+	+	−	−	−	−
25	" 62 (18th ").	++	++	+	+	−	−
62	" 62 (18th ")	++	++	++	++	+	−
11	Sm.73 (4th day).	−	−	−	−	−	−
12	" 73 (4th ").	−	−	−	−	−	−
14	" 73 (4th ").	−	−	−	−	−	−
15	" 73 (4th ").	−	−	−	−	−	−
24	" 73 (4th ").	−	−	−	−	−	−
25	" 73 (4th ").	−	−	−	−	−	−
R273	" 73 (4th ").	−	−	−	−	−	−
11	Sm.73 (18th day).	+	+	−	−	−	−
12	" 73 (18th ").	−	−	−	−	−	−
14	" 73 (18th ").	+	+	−	−	−	−
15	" 73 (18th ").	++	++	+	+	−	−
24	" 73 (18th ").	+	+	+	+	−	−
25	" 73 (18th ").	++	++	+	+	−	−
R273	" 73 (18th ").	++	+	+	−	−	−
80	Sm.203 (14th day).	−	−	−	−	−	−
81	" 203 (14th ").	−	−	−	−	−	−
82	" 203 (14th ").	−	−	−	−	−	−
94	" 203 (14th ").	++	+++	+++	+++	++	−
96	" 203 (14th ").	−	−	−	−	−	−
112	" 203 (14th ").	−	−	−	−	−	−
204	" 203 (14th ").	−	−	−	−	−	−
206	" 203 (14th ").	−	−	−	−	−	−
207	" 203 (14th ").	−	−	−	−	−	−
201	Sm.207 (15th day).	−	−	−	−	−	−
202	" 207 (15th ").	−	−	−	−	−	−
204	" 207 (15th ").	−	−	−	−	−	−
206	" 207 (15th ").	−	−	−	−	−	−

In Tables V to VIII, XI, XII, and XIV − indicates no agglutination, ± doubtful agglutination, + slight agglutination, ++ frankly positive agglutination, +++ heavy agglutination, ++++ complete agglutination.

streptococcus from a source other than scarlet fever. Similar results are illustrated by Tables VII and VIII.

The experiments shown in Tables VI to VIII show that it is possible to obtain a specific absorption of agglutinins from antiscarlatinal sera by use of streptococci from scarlet fever. Success, however, did not attend all such efforts and from time to time insurmountable difficulties are encountered.

Protection Tests.

An effort has been made to confirm the specificity of the agglutination reaction of the streptococci from scarlet fever by means of the protection test. Antistreptococcic sera as a rule have a rather low protective value for experimental animals in comparison, for example, with antipneumococcic sera. Previous investigators who have studied this question have reported in some instances a certain degree of specific protection and in others that the protective power of a monovalent serum is manifest against more than one type of streptococcus. In carrying out this test it is important that the serum should have a fairly high protective titer and that the organisms used should be of maximum virulence in order that any single test may extend over a sufficiently wide range of dosage. In the experiments shown in Tables IX and X the methods and conditions prescribed by Dochez, Avery, and Lancefield have been observed.

These experiments show that it is possible to develop in immune sera a certain degree of protective value for white mice against highly virulent strains of streptococcus isolated from scarlet fever. Such protective power is manifest not only against the homologous strain but also against strains from other cases of scarlet fever. Immune sera prepared by the use of strains of hemolytic streptococcus from sources other than scarlet fever possess no such protective power against scarlatinal streptococci.

Study of Contacts with Scarlet Fever.

It has long been recognized that scarlet fever may be propagated by means of carriers and does not require direct contact for its transmission. Sometimes the carrier may be a healthy individual, or may have suffered from a slight sore throat, without developing an exan-

TABLE VI.

Absorption Experiment with Scarlatinal Antistreptococcic Serum, No. 15a, and with Non-Scarlatinal Antistreptococcic Serum, No. 102.

Two specimens of 1 cc. each of Serum Sc.Sm.15a, 1:5, were absorbed with scarlatinal streptococcus, Strain 15 (homologous strain), and with non-scarlatinal streptococcus, Strain 102, respectively, as described above. Specimens of Non-Scarlatinal Serum 102 were similarly absorbed. The experiment follows.

Row.	Strepto-coccus strains.	Sera.	Dilution.					
			1:40	1:80	1:160	1:320	1:640	1:1,280
1	15	Sc.Sm.15a absorbed with Scarlatinal Strain 15.	−	−	−	−	−	−
2	15	" " 15a " " Non-Scarlatinal Strain 102.	+	++++	++++	++++	+++	+++
3	15	Non-Sc.Sm.102 absorbed with Scarlatinal Strain 15.	−	−	−	−	−	−
4	15	" " 102 " " Non-Scarlatinal Strain 102.	−	−	−	−	−	−
5	15	Normal rabbit serum.	−	−	−	−	−	
	15	Plain broth control.						−
6	201	Sc.Sm.15a absorbed with Strain 15.	−	−	−	−	−	−
7	201	" " 15a " " " 102.	++	++	+	+	−	−
8	201	Non-Sc.Sm.102 absorbed with Strain 15.	−	−	−	−	−	−
9	201	" " 102 " " " 102.	−	−	−	−	−	−
10	201	Normal rabbit serum.	−	−	−	−	−	
	201	Plain broth control.						−
11	112	Sc.Sm.15a absorbed with Strain 15.	++	+	−	−	−	−
12	112	" " 15a " " " 102.	++	++	++	++	++	+
13	112	Non-Sc.Sm.102 absorbed with Strain 15.	−	−	−	−	−	−
14	112	" " 102 " " " 102.	−	−	−	−	−	−
15	112	Normal rabbit serum.	−	−	−	−	−	
	112	Plain broth control.						−

16	102	Sc.Sm.15a absorbed with Strain 15.	−	−	−	−	−	−
17	102	" " 15a " " " 102.	±	±	−	−	−	−
18	102	Non-Sc.Sm.102 absorbed with Strain 15.	++	++	++	+	−	−
19	102	" " 102 " " " 102.	−	−	−	−	−	−
20	102	Normal rabbit serum.	−	−	−	−	−	
	102	Plain broth control.						−
21	114	Sc.Sm.15a absorbed with Strain 15.	−	−	−	−	−	−
22	114	" " 15a " " " 102.	−	−	−	−	−	−
23	114	Non-Sc.Sm.102 absorbed with Strain 15.	−	−	−	−	−	−
24	114	" " 102 " " " 102.	−	−	−	−	−	−
25	114	Normal rabbit serum.	−	−	−	−	−	
	114	Plain broth control.						−

Strains 15 and 201 were isolated from scarlatinal throats, No. 112 from the probable carrier referred to above, and Nos. 102 and 114 from throats in cases of tonsillitis.

TABLE VII.

Absorption Experiment with Scarlatinal Antistreptococcic Serum, No. 76a.

Two specimens of 1 cc. each of scarlatinal antistreptococcic serum, No. 76a, were absorbed with scarlatinal streptococcus, Strain 15, and with non-scarlatinal streptococcus, Strain 102, respectively.

Row.	Streptococcus strains.	Sera.	Dilution.					
			1:40	1:80	1:160	1:320	1:640	1:1,280
1	15	Sc.Sm.76a absorbed with Scarlatinal Strain 15.	−	−	−	−	−	−
2	15	" " 76a " " Non-Scarlatinal Strain 102.	+++	+++	+++	+++	+	−
3	15	" " 76a not absorbed.	++++	++++	++++	++++	++++	++
4	15	Normal rabbit serum.	−	−	−	−	−	
	15	Plain broth control.						−
5	201	Sc.Sm.76a absorbed with Scarlatinal Strain 15.	−	−	−	−	−	−
6	201	" " 76a " " Non-Scarlatinal Strain 102.	+++	+++	+++	+	−	−
7	201	" " 76a not absorbed.	++++	++++	++++	++	+	−
8	201	Normal rabbit serum.	−	−	−	−	−	
	201	Plain broth control.						−
9	112	Sc.Sm.76a absorbed with Scarlatinal Strain 15.	−	−	−	−	−	−
10	112	" " 76a " " Non-Scarlatinal Strain 102.	+++	+++	+++	++	+	−
11	112	" " 76a not absorbed.	++++	++++	++++	++	+	−
12	112	Normal rabbit serum.	−	−	−	−	−	
	112	Plain broth control.						−
13	117	Sc.Sm.76a absorbed with Scarlatinal Strain 15.	−	−	−	−	−	−
14	117	" " 76a " " Non-Scarlatinal Strain 102.	++	+	+	−	−	−
15	117	" " 76a not absorbed.	+++	+++	+++	+	−	−
16	117	Normal rabbit serum.	−	−	−	−	−	
	117	Plain broth control.						−

17	102	Sc.Sm.76a absorbed with Scarlatinal Strain 15.	−	−	−	−	−	−
18	102	" " 76a " " Non-Scarlatinal Strain 102.	−	−	−	−	−	−
19	102	" " 76a not absorbed.	−	−	−	−	−	−
20	102	Normal rabbit serum.	−	−	−	−	−	
	102	Plain broth control.						−
21	114	Sc.Sm.76a absorbed with Scarlatinal Strain 15.	−	−	−	−	−	−
22	114	" " 76a " " Non-Scarlatinal Strain 102.	−	−	−	−	−	−
23	114	" " 76a not absorbed.	+	−	−	−	−	−
24	114	Normal rabbit serum.	−	−	−	−	−	
	114	Plain broth control.						−

Strains 15 and 201 were isolated from the throats of scarlet fever patients, Nos. 112 and 117 from the throat of the carrier, and Nos. 102 and 114 from throats in cases of tonsillitis.

TABLE VIII.

*Absorption Experiment with Scarlatinal Antistreptococcic Serum, No. 15a, Absorbed with Scarlatinal Carrier Streptococcus, Strain 112, and Non-Scarlatinal Streptococcus, Strain 114.**

Row.	Strepto-coccus strains.	Sera.	Dilution.					
			1:40	1:80	1:160	1:320	1:640	1:1,280
1	15	Sc.Sm.15a absorbed with scarlet fever carrier strain, No. 112.	−	−	−	−	−	−
2	15	" " 15a " " Non-Scarlatinal Strain 114.	+++	+++	+++	+	−	−
3	15	" " 15a not absorbed.	++++	++++	++++	++++	+++	+++
4	15	Normal rabbit serum.	−	−	−	−	−	
	15	Broth.						−
5	112	Sc.Sm.15a absorbed with scarlet fever carrier strain, No. 112.	−	−	−	−	−	−
6	112	" " 15a " " Non-Scarlatinal Strain 114.	+	++	+	+	−	−
7	112	" " 15a not absorbed.	++	++	++	++	+	+
8	112	Normal rabbit serum.	−	−	−	−	−	
	112	Broth.						−
9	114	Sc.Sm.15a absorbed with scarlet fever carrier strain, No. 112.	−	−	−	−	−	−
10	114	" " 15a " " Non-Scarlatinal Strain 114.	−	−	−	−	−	−
11	114	" " 15a not absorbed.	−	−	−	−	−	−
12	114	Normal rabbit serum.	−	−	−	−	−	
	114	Broth.						−

*The same technique was used as in Table VII.

TABLE IX.

Protection against Virulent Scarlatinal Streptococcus, Strain 25, with Antistreptococcic Sera C13 and 53.*

The mice were inoculated in the afternoon of the 1st day intraperitoneally with 0.5 cc. each of the sera, and on the morning of the 2nd day with the graduated doses of the virulent streptococcus, No. 25, as indicated.

Mouse No.	Serum.	Culture.	Result.
		cc.	
1	None.	0.0001	Died in 30 hrs.
2	"	0.00001	" " 36 "
3	"	0.000001	Very sick for 2 days, but recovered.
4	0.5 cc. of Serum Sm.C13.	0.001	Died in 36 hrs.
5	0.5 " " " " C13.	0.0001	Survived.
6	0.5 " " " " C13.	0.00001	"
7	0.5 " " " " C13.	0.000001	"
8	0.5 " " " " 53.	0.001	"
9	0.5 " " " " 53.	0.0001	"
10	0.5 " " " " 53.	0.00001	"
11	0.5 " " " " 53.	0.000001	"

*Strain 25 had been so raised in virulence through twenty-eight mouse passages that less than 0.00001 cc. of a blood broth culture killed a mouse in less than 48 hours.

TABLE X.

Protection against Virulent Scarlatinal Streptococcus, Strain 25, with Scarlatinal Antistreptococcic Sera, Nos. 15 and 76.*

Mouse No.	Serum.	Culture.	Result.
		cc.	
1	None.	0.0001	Died in 72 hrs.
2	"	0.00001	" " 36 "
3	"	0.000001	" " 48 "
4	0.5 cc. of Serum Sm.15.	0.01	" " 24 "
5	0.5 " " " " 15.	0.001	Survived.
6	0.5 " " " " 15.	0.0001	Died in 70 hrs. (Peritoneal fluid sterile.)
7	0.5 " " " " 15.	0.00001	Died in 76 hrs.
8	0.5 " " " " 76.	0.01	" " 72 "
9	0.5 " " " " 76.	0.001	Survived.
10	0.5 " " " " 76.	0.0001	Died in 28 hrs.
11	0.5 " " " " 76.	0.00001	Survived.

*The strain and the technique used were the same as in Table IX.

them. An unusual opportunity to study such a set of conditions was afforded by a localized outbreak of scarlet fever in a practically isolated community.[1]

Beyond the outskirts of the little village of Claiborn, on the east shore of the Chesapeake Bay, the Maryland Tuberculosis Association maintains a fresh air home, called Claiborn Cottage, where non-tuberculous children of tuberculous parents, children below par physically, but with no physical signs of tuberculosis, are sent for several weeks or months during the summer. The cottage is situated directly on the shore of the bay and is surrounded on the remaining sides by dense woods. No parents or friends visit the cottage. At the time of the outbreak, there were in the cottage thirty-three children, varying from 3 to 12 years of age, and two nurses and four colored helpers. On September 21, 1920, Wm. W., age 8, was taken ill with general malaise and a slight sore throat. He was immediately isolated. Later headache, vomiting, red throat, and flushing of the skin developed. On the morning of the 4th day (September 24) he exhibited a typical scarlatiniform erythema over the face, chest, and body. He was then transferred to the Sydenham Hospital. 2 days later (September 26) Edw. M., age 4, was taken ill with vomiting. He, too, was promptly quarantined in a separate room. 2 days later he developed a typical angina and strawberry tongue. Simultaneously, on September 26, Jas. L., age 12, also became ill, and complained of headache and slight sore throat. He was also quarantined and 2 days later showed a typical scarlatinal rash. Edw. M. and Jas. L. were then sent to Sydenham Hospital, and on the day following, Edw. M. exhibited a scarlatiniform rash. All three patients subsequently experienced typical attacks of uncomplicated scarlet fever, followed by desquamation. The author visited the three boys at the Sydenham Hospital and isolated strains of hemolytic streptococcus from the throats of all. No other case of scarlet fever developed at the Claiborn Cottage.

The question then arose as to the source of infection. It could not have been the neighboring village, since the children were never allowed to leave the premises nor did the villagers ever visit the cottage. Even the servants lived in a small cottage on the grounds. Furthermore, there were no cases of scarlet fever reported in that county at the time. The milk supply was beyond suspicion, inasmuch as it came from one small farm nearby, and was handled by only the farmer's immediate family and the staff of the Claiborn Cottage. Careful investigation failed to reveal any history of sore throat, or any indisposition whatsoever among any of the members of the farmer's family or help. The infection was not introduced by visitors since there had been no visitors during the summer. Suspicion was directed, therefore, to the only recent admissions to the cottage, three little

[1] For this opportunity I am indebted to Dr. John F. Hogan of the Baltimore Department of Health, and to Miss Spielman of the Maryland Tuberculosis Association.

brothers, Joseph, Francis, and James B., Russian-Americans, who had been admitted to Claiborn Cottage, September 17, 4 days before the development of the first case of scarlet fever. There had been no other admissions within 3 weeks.

Joseph, Francis, and James B., age 11, 10, and 8 years, at the time of observation were apparently free from any acute infection, and denied having had sore throats recently. However, at the time they left home, September 17, a sister, Julia B., age 12, was ill with a severe sore throat, which had lasted for several days. She visited on September 20 the children's dispensary of the Johns Hopkins Hospital, where it is stated on her record that she had just recovered from an acute tonsillitis. No note was made at that time of the presence of a rash or of desquamation, nor could a history of these be obtained later from the mother.

The cottage was visited by the author on October 1 and cultures were taken on blood agar plates from the throats of the thirty-nine inhabitants. From the throats of twenty-three, including the nurses and helpers, no hemolytic streptococci were recovered. On the other hand, hemolytic streptococci were obtained in greater or less numbers from sixteen of the inmates of the cottage, the largest numbers of colonies being present on the plates from James B., Joseph B., and Francis B., the three recent admissions. None of the latter at any time had sore throat, fever, or rash. An immune serum, No. C13, was prepared by immunization of a rabbit with Strain C13 from Joseph B. The capacity of this serum to agglutinate the group of Claiborn streptococci was then tested. The results of this test are shown in Table XI.

The result of this series of tests demonstrates that none of the strains of hemolytic streptococci isolated from the throats of the children in the Claiborn Cottage was agglutinated by the serum made by immunization of a rabbit to one of these strains (No. C13), except the homologous strain, and also (slightly) Strain C15 (Francis B.). On the other hand, this serum agglutinated seventeen out of twenty strains of scarlatinal streptococci (Table XII). It is of particular interest to note that among these scarlatinal strains were Nos. 76, 77, and 78, the strains isolated from the throats of Wm. W., Edw. M., and Jas. L., the boys who had contracted scarlet fever while at Claiborn Cottage.

Furthermore, the Claiborn strains, with the exception of Nos. C13 and C15, and the irregularly agglutinated Strains C9 and C34, failed to be agglutinated by five scarlatinal antistreptococcic sera. The results of this test are shown in Table XIII.

The series of tests shown in Tables XI to XIII would seem to indicate that hemolytic streptococcus strain, No. C13, carried in the throat of Joseph B. differed from all others found in the throats of the

children at Claiborn, except that from his brother Francis B., Strain C15. Study of its agglutination reactions shows it to be of the same biological type as strains isolated from the great majority of throats in scarlet fever, indicating that it is a true scarlatinal streptococcus.

TABLE XI.

Agglutination of Hemolytic Streptococci from the Throats of Inhabitants of Claiborn Cottage by Serum C13.

Streptococcus strains.	Dilution of Antistreptococcic Serum C13.					
	1:40	1:80	1:160	1:320	1:640	1:1,280
C1	−	−	−	−	−	−
C7	−	−	−	−	−	−
C8	−	−	−	−	−	−
C9	−	−	−	−	−	−
C10	−	−	−	−	−	−
C13	++++	++++	+++	+++	+++	+++
C15	++	+	+	−	−	−
C18	−	−	−	−	−	−
C19	−	−	−	−	−	−
C20	−	−	−	−	−	−
C21	−	−	−	−	−	−
C22	−	−	−	−	−	−
C23	−	−	−	−	−	−
C30	−	−	−	−	−	−
C33	−	−	−	−	−	−
C34	−	−	−	−	−	−

The throats were numbered serially, as cultured, so that the above numbers represent the positive cultures from the thirty-nine throats examined.

Immune sera were also made with Strain 76, the strain isolated from Wm. W., the first case of scarlet fever to develop at Claiborn Cottage. These sera, Sm.76a, and Sm. 76b, agglutinated typically streptococci isolated from other scarlatinal throats. The results have already been tabulated in Table III. They also agglutinated Strain C13 in high dilution, but failed to agglutinate the other Claiborn strains. In short, Serum 76 gave identically the same agglutination reactions as did Serum C13.

A culture was made from the throat of Julia B., the sister of the three boys, and although 2 weeks had elapsed since her attack of tonsillitis, a few colonies of

hemolytic streptococcus were obtained. Strain 79 isolated from this source was agglutinated by one antiscarlatinal serum but not by two other such sera. As the strain quickly became persistently granular, this discrepancy could not be further investigated.

A fourth brother, Louis B., age 9, came to the Johns Hopkins dispensary, November 17, giving a history of having had a cold about October 1, just after

TABLE XII.

Agglutination of Scarlatinal Streptococcus Strains by Serum C13.

Scarlatinal streptococcus strains.	Dilution of Serum C13.					
	1:40	1:80	1:160	1:320	1:640	1:1,280
74	++++	++++	++++	++	+	−
75	++++	++++	++++	++	−	−
76	++++	++++	++++	++++	++++	++++
77	++++	+++	+++	++	++	+
78	++++	++++	++++	++++	++++	++++
79	++	++	++	++	++	++
80	++++	++++	++++	++++	+++	+
81	++++	+++	++	+	−	−
82	++++	++++	++++	++	−	−
83	−	−	−	−	−	−
84	++++	++++	++++	++	+	−
88	+++	+++	+++	++	++	+
89	++++	++++	++++	++++	+++	++
90	+++	++	++	++	+	−
91	+++	++	+	−	−	−
93	++++	++++	++++	++++	+++	+++
94	−	−	−	−	−	−
95	−	−	−	−	−	−
98	+++	+++	++	+	−	−
99	++	++	++	++	+	−

the sister's recovery from sore throat and while the other three brothers were at Claiborn Cottage. His mother noticed that his face was swollen, and that during the following week the swelling extended to his extremities and abdomen. The patient had no angina, rash, or subsequent desquamation of the skin. About October 8, the edema became very marked and he began to have fever and headache. He was kept in bed 2 weeks, during which time the swelling gradually disappeared. The mother then noticed urinary frequency, and on November 17,

the patient was brought to the Johns Hopkins Hospital dispensary. At this time his feet were still a little edematous, and his urine showed a trace of albumin and some casts. His tonsils were enlarged, and cultures from his throat on a blood agar plate yielded a considerable number of colonies of hemolytic streptococcus. The strain isolated is designated C40. The agglutination reactions of this strain are shown in Table XIV.

TABLE XIII.

Agglutination of Claiborn Contact Streptococcus Strains with Scarlatinal Antistreptococcic Sera.

Claiborn contact strains.	Scarlatinal antistreptococcic sera.				
	15a.	15b.	25.	53a.	76a.
C1	−	−	−	−	−
C7	−	−	−	−	+
C8	−	−	−	−	−
C9	++++++	+++++	−	+++	+++++
C10	++	−	−	−	−
C13	+++	+++	+++	+++++	++++++
C15	++++	+++	−	+	−
C18	−	−	−	−	−
C19	−	−	−	−	++++
C20	−	−	−	−	−
C21	−	−	−	−	−
C22	−	−	−	−	−
C23	−	−	−	−	−
C30	−	−	−	−	−
C33	−	−	−	−	−
C34	++++++	++++	+	−	−

Table XIV shows Strain C40 to be closely related to Strain C13, isolated 2 weeks previously from the throat of the patient's brother, and to Strain 76, isolated from one of the cases of scarlet fever which developed at Claiborn Cottage. The failure of other scarlatinal antistreptococcic sera tested to agglutinate Strain C40 is an irregularity which cannot be satisfactorily explained at the present time.

A second study of contacts was made in connection with a case of scarlet fever developing among the women students of the Johns Hopkins Medical School. Immediately after the development of

scarlet fever by the individual mentioned, throat cultures were taken of all the women students frequenting the club hall in which the patient lived, as well as of four men students working in close proximity to her in the anatomy dissecting room. The throats of fifty-

TABLE XIV.

Agglutination of Possible Scarlatinal Contact Streptococcus Strain, No. C40, with Scarlatinal Antistreptococcic Sera.

Sera.	Dilution.					
	1:20	1:40	1:80	1:160	1:320	1:640
15a	−	−	−	−	−	−
15b	−	−	−	−	−	−
25	−	−	−	−	−	−
53a	−	−	−	−	−	−
53b	−	−	−	−	−	−
76a	++	++	++	++	+	+
76b	+++	+++	+++	++	++	+
C13	++++	++++	++++	++++	++++	++++

TABLE XV.

Agglutination of a Series of Scarlatinal Contact Streptococcus Strains with Scarlatinal Antistreptococcic Sera.

Contact streptococcus strains.	Scarlatinal antistreptococcic sera.			
	Sm.15a.	Sm.25.	Sm.53a.	Sm.76a.
D1	−	−	−	−
D2	−	−	−	−
D3	−	−	−	−
D4	−	−	−	−
D5	−	−	−	−
D6	−	−	−	−
D7	−	−	−	−
D8	−	−	−	−
D9	−	−	−	−
D10	++++++	+++	−	++++++

three persons were cultured, thirty-five of whom had been in close contact with the patient shortly before she developed scarlet fever. Of the total number of throats cultured ten showed hemolytic streptococci. The results of agglutination tests of these ten strains are recorded in Table XV.

From but one individual in the series was a hemolytic streptococcus isolated having agglutinating properties similar to those observed among streptococci isolated from scarlet fever. None of the contacts subsequently developed scarlet fever.

SUMMARY.

1. Hemolytic streptococcus has been found in 100 per cent of the throats of patients with scarlet fever during the 1st week of the disease.

2. The average length of time that these organisms are present in the throat varies from 10 to 20 days.

3. No morphological or cultural characteristics peculiar to the hemolytic streptococcus from scarlet fever can be demonstrated.

4. Ten immune sera have been prepared from different strains of scarlet fever streptococci and each of the sera agglutinated more than 80 per cent of the strains isolated from scarlatinal throats. On the other hand, scarlatinal streptococci are not agglutinated by immune sera prepared from hemolytic streptococci isolated from other pathological sources.

5. Serum from patients convalescent from scarlet fever agglutinates weakly or not at all the homologous strain of hemolytic streptococcus.

6. The specificity of the agglutination reaction of scarlatinal streptococci is confirmed by absorption experiments.

7. Scarlatinal antistreptococcic serum affords some degree of protection against virulent scarlet fever streptococci but has no protective power against hemolytic streptococci from other diseases.

8. In a small epidemic of scarlet fever a healthy carrier of hemolytic streptococcus was detected; the organism carried was identical in its serological reactions with strains of hemolytic streptococci isolated from active cases of scarlet fever.

9. In a study of a number of contacts with a case of scarlet fever, in only one instance was a scarlatinal type of hemolytic streptococcus recovered from the throat.

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